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13. ABSTRACT (Maximum 200 Words)

We selected *Saccharomyces cerevisiae* mutants from the diploid deletion collection that suppressed G1 arrest and lethality following heterologous expression of BRCA1. Expression of BRCA1 was confirmed by immunofluorescence or Western blot analysis. We also screened the diploid deletion strains for altered sensitivity to the transcription inhibitor zymocin to identify those that also suppress BRCA1-induced lethality and are radiation sensitive. We identified conserved components of the CCR4 damage response network and many of these genes, including *CCR4, DHH1, DEF1, HCM1, SPT4, SPT5, SUB1, YAF9, YAP3* and numerous components of the nuclear pore complex are required for transcription. These genes confer resistance to radiation and the transcription elongation inhibitor zymocin. We hypothesize that BRCA1 and zymocin stall transcription elongation in G1. When cells progress into S phase, the stalled transcription complexes serve as replication blocks that are processed into lethal DNA double-strand breaks. Consistent with this model is the observation that BRCA1 enhanced plasmid degradation and loss in WT as compared to *SPT4* deleted cells. Using co-immunoprecipitation (but not two hybrid analysis), we determined that Spt4p (and Dhh1p) physically interact with BRCA1 in a complex while the highly conserved human orthologue of Dhh1p (DDX6) physically interacts with BRCA1 in human cells.

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Introduction: In hereditary breast cancer, changes in DNA repair ability have been identified that result from alterations in the BRCA1 and BRCA2 gene products. These genes have been found to be pleiotropic tumor suppressors that impact on recombinational repair of DSB damage, cell cycle checkpoint arrest following DNA damage, transcriptional regulatory mechanisms, and centrosome duplication (16) (28) (39) (38). BRCA1 also appears to be involved in multiple transcriptional functions including the regulation of p21, p53, Mdm2 and Gadd45 (24) (16) (10). Furthermore, BRCA1 is known to bind to the RNA pol II holoenzyme complex (32) (1). A growing body of evidence suggests that BRCA1 may participate as a key component in a novel genome surveillance mechanism by associating with hyperphosphorylated forms of RNA pol II (19) that can signal the presence of DNA damage when elongating RNA pol II complexes encounter DNA lesions (reviewed in (22) (20)). Furthermore, BRCA1 can modulate phosphorylation of the RNA pol II carboxy-terminal domain (CTD) (27) which appears to play a critical role in transcriptional elongation. Interestingly immunoprecipitation of BRCA1 co-purifies with active forms of RNA pol II and TFIIH (1) which itself plays multiple roles in DNA repair (11).

Recently, BRCA1 has been found to bind through one of the BRCT domains to a novel *co* factor of *B*RCA1 (COBRA1) which in turn binds to a specific genomic site to promote chromatin decondensation (40). COBRA1 has been found as an integral component of the human *n*egative *el*ongation *f*actor (NELF) that binds to *DRB* (5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole) *s*ensitivity *i*nducing *f*actor (DSIF) to cause transcriptional pausing of RNA pol II holoenzyme complexes (29). Not surprisingly, DSIF has been found to be required for transcriptional elongation by RNA pol II and is composed of the human orthologues of the yeast Spt4p and Spt5p (34). Therefore, our finding that deletion of *SPT4* rescues BRCA1 induced G1 arrest and lethality in yeast and that BRCA1 physically binds Spt4p in yeast (see Appendix A below) suggests that BRCA1 may similarly bind human Spt4 in human cells to arrest transcriptional elongation. This activity is predicted to be particularly robust at G1 in yeast since this is a period of intense transcriptional activity where many S phase specific genes are induced in a cell cycle specific manner (14).

Our combined data suggests a molecular model where BRCA1 binds to the highly conserved human Spt4 protein to regulate transcriptional arrest when RNA pol II encounters DNA damage in human cells. Thus the major role of BRCA1 in damage signaling may be mediated through a transcriptional damage sensor mechanism. A similar role has been suggested for p53 activation in response to bulky UV lesions encountered by the transcriptional apparatus (26) (23). Further evidence supporting a role for BRCA1 in the elongation step of mRNA sysnthesis has been suggested by the physical association of BRCA1 with NUFIP; a factor that stimulates transcription by association with the positive elongation factor P-TEFb and the regulatory cyclin T subunit (7). Taken together, these results have allowed us to synthesize a molecular model for the mechanism of radioresistance among members of the CCR4 damage response network in yeast. This network is highly conserved and essential features of this model integrate the biological effects we have observed following heterologous expression of BRCA1 in yeast.

We have used the yeast *Saccharomyces cerevisiae* to identify new, highly conserved genetic targets that interact with the breast cancer tumor suppressor protein

BRCA1. As described above, BRCA1 is known to physically interact in human cells with a large number of proteins that have functions related to transcription, chromatin remodeling, centrosome duplication and a variety of DNA repair processes. In order to identify a highly conserved set of BRCA1 interacting genes that define a basic primary function for the BRCA1 tumor suppressor, we used the genetically accessible eukaryotic model organism *S. cerevisiae* in a genetic screen for altered BRCA1 functions. Briefly, we expressed BRCA1 from an inducible promoter on a high copy selectable plasmid in WT diploid cells, which resulted in a pronounced G1 arrest and lethality. We hypothesize that lethality results from the inappropriate interaction of BRCA1 in a complex with highly conserved proteins that have functions essential for both the maintenance of genetic stability (*i.e.* DNA repair or checkpoint functions) and yeast cell survival. We therefore transformed a large pool of isogenic diploid deletion strains to identify yeast deletions that suppressed (or rescued) BRCA1-induced lethality following heterologous expression in yeast. A number of highly conserved deletion mutants that regulate transcription including *CCR4, DHH1, SPT4, SPT5, YAF9, HCM1, YAP3* and numerous components of the nuclear pore complex have been identified to suppress BRCA1-induced G1 arrest and lethality.

Many of the transcription regulators found to suppress BRCA1 induced lethality have also been shown to exhibit enhanced sensitivity to ionizing radiation (IR) as well as the lethal effects of the toxin zymocin secreted by the yeast *Kluyveromyces lactis*. In yeast, lethal IR-induced DNA damage is thought to result from the persistence of unrepaired DNA double-strand breaks (DSBs). IR-induced DSBs are repaired almost exclusively by recombination, which requires the activities of the *RAD52* epistasis group of repair genes and an undamaged DNA homolog to template the recombinational repair event. Since yeast cells contain little repetitive DNA, irradiation of haploid repair competent yeast cells in G1 is extremely lethal. However, haploid yeast cells irradiated in G2 are radioresistant since they can repair DSB damage using the newly replicated sister chromatid as a template for recombinational repair. On the other hand diploid repair competent yeast cells irradiated in G1 are radioresistant and survive since they can undergo recombinational repair using the undamaged homolog. Using epistasis analysis, *CCR4* has been found to mediate resistance to IR in diploid yeast cells as a member of the *RAD9* checkpoint repair pathway (36). Furthermore, deletion of *CCR4* or *DHH1* in haploids does not result in enhanced IR sensitivity when compared to WT clearly indicating that the diploid population of G1 cells is radiosensitive. IR sensitivity of the diploid *ccr4Δ* does not appear to result from a decrease in the ability to undergo recombination, instead *CCR4* (and *DHH1*) appear to be required for reentry into the cell cycle following IR mediated DNA damage checkpoint arrest in G1 (36).

Deletion of *CCR4* in diploid as well as haploid cells results in sensitivity to the S phase specific DNA damaging agents hydroxyurea (HU) and methyl methanesulfonate (MMS). This sensitivity was separate from that seen for diploid *rad52Δ* cells since diploid *rad52Δccr4Δ* double mutants were more sensitive to HU than the either the isogenic *rad52Δ* or *ccr4Δ* mutants alone (36). When G1 *ccr4Δ* cells from a logarithmic phase population were exposed to HU, the cells progressed from G1 into S phase and arrested as budded (G2) cells, which eventually lysed. This lethal S/M arrest phenotype was not seen for WT diploid cells and clearly indicates that *ccr4Δ* cells have a checkpoint defect(s) that extend into S phase. This checkpoint defect has also been observed for

ccr4Δ cells following exposure to zymocin, a lethal toxin that specifically inhibits transcription in susceptible yeast strains by binding to components of the RNA pol II elongation complex (35). We have observed that most of the deletion mutants found to be sensitive to IR including members of the *RAD52, RAD6* and *RAD9* epistasis groups are also sensitive to zymocin. Furthermore, IR sensitive deletion mutants with known defects in replication related functions such as *mms22Δ,* are hypersensitive to the lethal effects of zymocin (35). These genetic results and physical plasmid DNA degradation studies following zymocin exposure or BRCA1 induction (see below) indicate that both conditions appear to induce DSB damage.

Taken together, we have developed a molecular model for zymocin and BRCA1-induced lethality in yeast that can accomidate the above results. In this model, BRCA1 and zymocin inhibit transcription elongation that elicits a G1 arrest in WT diploid cells. Eventually these cells adapt to the cell cycle checkpoint signal generated by the arrested RNA pol II transcription complexes and enter S phase where progression of replication forks are blocked when they encounter the stalled RNA pol II holoenzyme complexes. These replication blocks are poorly repaired and are subsequently processed by nucleolytic enzymes into replication-induced DSBs. Under normal conditions, cells produce spontaneous DNA damage from a variety of sources including oxidative metabolic processes that produce free radicals and single-strand DNA breaks (SSBs). It should be noted that IR also produces free radical induced DNA damage in the form of SSBs and that this form of damage is extensive since SSBs outnumber DSBs 20:1 following IR exposure in diffusible water environments (4). Thus we predict that the CCR4 network of radiation response genes are required for the proper signaling and/or repair of RNA pol II holoenzyme complexes stalled by DNA damage including IR-induced SSBs. Therefore, *ccr4Δ* cells are predicted to be hypersensitive to agents such as hydrogen peroxide (H2O2) that induce predominantly free radical mediated SSB damage. This appears to be the case (see Fig. 2 below) as *ccr4Δ* cells exhibit enhanced sensitivity to oxidative damage. Given the requirement for BRCA1 function in resistance to oxidative damage, we further predict that BRCA1 functions in concert with the highly conserved Ccr4p (and other transcription regulators described above) to signal and/or repair RNA pol II holoenzyme complexes stalled by oxidative DNA damage. Following is the progress we have made in year two of this grant in support of this hypothesis.

Body: *Task 1.* Identify and characterize in yeast, genes that rescue BRCA1-induced slow growth and lethality. (Months 1-24)

a. Finalize screening of the identified IR^S diploid deletion strains (from Bennett et al., 2001; and unpublished) and identify new deletion mutants from a random pool of deletion mutants for those that rescue slow growth and lethality following BRCA1 expression. Collect~100 deletion mutant clones from pool for sequencing identification (Months 1-12).

This task has been completed. Additional deletion mutants have been added to the list of those that rescue BRCA1-induced lethality in yeast (see Tables 1 and 2; year 1 annual report). These include the ionizing radiation (IR) resistance gene *DEF1* (formerly *VID31*) and numerous components of the nuclear pore complex. Deletion of the nuclear

pore components Nup2 and Pom34 were previously found to suppress BRCA1-induced lethality from our screen of the random pool of deletion mutants (see Table 2 within year 1 annual report). Subsequently, deletions of other nuclear pore components including *ASM4, NUP53, NUP84, NUP120, NUP133, NUP170* and *NUP188* were also found to suppress BRCA1-induced lethality (Fig. 1A).

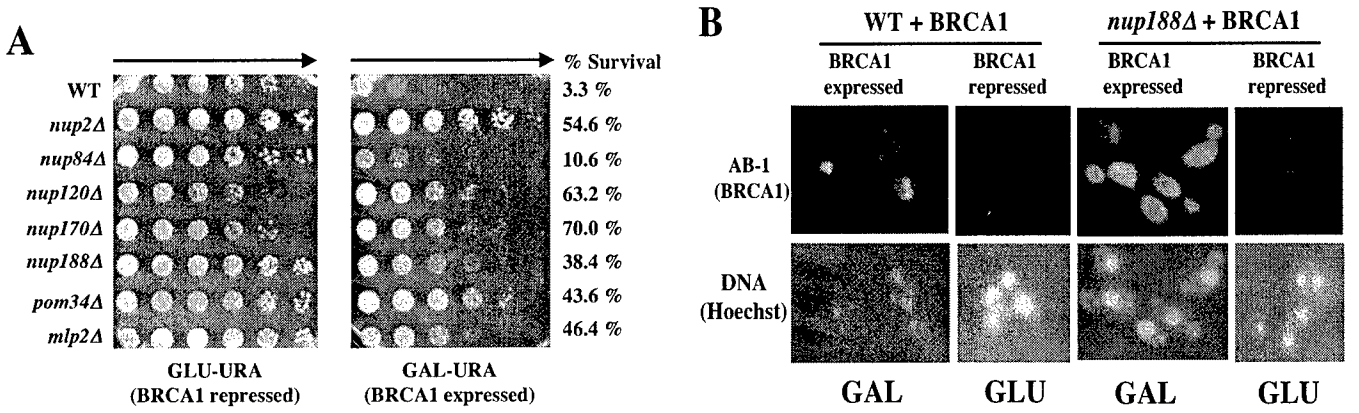

Fig. 1. Nuclear pore mutants suppress BRCA1 induced lethality. Panel A: Diploid deletion strains defective in nuclear pore functions transformed with the *GAL*::BRCA1 plasmid were grown and replica plated to synthetic complete (SC) glucose (GLU) – uracil (URA) (BRCA1 repressed) and SC galactose (GAL) – URA (BRCA1 expressed) . Percent (%) survival was calculated as the relative plating efficiencies for colony forming ability of cells plated to SC GAL-URA *vs* SC GLU-URA. Panel B: WT and strains deleted for components of the nuclear pore were transformed with the *GAL*::BRCA1 plasmid and induced for BRCA1 expression for 6 hours in SC GAL-URA medium. Cells were fixed in formaldehyde, made permeable with zymolyase and stained with anti-BRCA1 mouse monoclonal antibody (AB-1). Cells were incubated with goat anti mouse secondary antibody conjugated to Alexa Fluor 488. All nuclear pore mutants depicted in panel A expressed BRCA1 at levels equal to or greater than WT. BRCA1 protein distribution was both cytoplasmic and nuclear. Nuclear position was determined by visualization of chromosomal DNA following staining with Hoechst 33342. The *nup188Δ* strain is a typical example of BRCA1 expression and protein localization in nuclear pore deficient strains.

These gene deletions were screened for suppression of BRCA1-induced lethality based on published genetic or physical interactions with Nup2p and Pom34p (see SGD website and links within) as well as previously identified sensitivity to IR (3) (36). Since the nuclear pore regulates molecular export and import functions for the nucleus, we examined whether BRCA1 protein expression and/or localization was affected following deletion of nuclear pore genes that are required for BRCA1 induced lethality. We therefore developed an *in situ* fixation protocol followed by indirect immunofluoresence protocol to examine the galactose induced expression of BRCA1 within individual WT and mutant yeast cells (Fig. 1B). We determined that the nuclear pore mutants (and the *spt4Δ* strain see below) all produced BRCA1 at levels similar to or greater than that observed for WT using *in situ* fixation and indirect IF staining following galactose-induced BRCA1 expression (Fig. 1B and see Fig. 4B, Appendix A). BRCA1 was not detected by indirect IF in WT or nuclear pore deficient strains that were grown in glucose (BRCA1 repressed) prior to fixation (Fig 1B).

Based on our identification that the diploid *ygr064wΔ* strain (which partially overlaps *SPT4*) rescued BRCA1-induced lethality (see Table 2 within year 1 annual

report), we further established that a null deletion of *SPT4* and two separate C terminal truncations of the *SPT5* gene each completely suppressed BRCA1-induced lethality. Furthermore, we characterized a dose-dependent increase in IR sensitivity for the diploid *spt4Δ* and *spt5* C terminal truncation when compared to WT using survival curve analysis. Since Spt4p and its cognate interactive partner Spt5p clearly mediate transcription elongation, complete suppression of BRCA1-induced lethality by inactivating these highly conserved proteins suggests that the transcription elongation complex may be the primary interactive target for BRCA1-induced toxicity in yeast. These results describing the genetic and physical interaction of Spt4p with BRCA1 in yeast as well as the sensitivity of the *spt4Δ* strain to hydroxyurea HU and methyl methane sulfonate (MMS) have been fully described in the accompanying manuscript that has been submitted for publication and is under review (see Appendix A).

b. Screen rescued yeast deletion clones from pool (*i.e.* those that do not show inhibited growth following BRCA1 expression) for radiation sensitivity. Those that show sensitivity to IR will be analyzed in detail by dose response curve analysis. Those that are not sensitive to IR will be tested for sensitivity to other DNA damaging agents such as UV, bleomycin, doxorubicin, camptothecin, mitomycin C as well as for spontaneous genomic instability resulting in chromosome loss. (Months 6-12).

Among the deletion strains identified to date, suppression of BRCA1-induced lethality was found to be the greatest for the *spt4Δ* strain when compared to WT. Furthermore, this gene (*SPT4*) has been the subject of numerous publications defining its role in yeast transcription elongation. Since this protein is also highly conserved and transcription elongation has been implicated as a possible target for BRCA1 interaction *i.e.* interaction with the human protein COBRA1 (29), we chose to concentrate much of our effort to examine Spt4p as a putative new target that would validate our genetic screen. As described above, we have analyzed the dose response of the yeast *spt4Δ* and *spt5* truncation strains to IR using survival curve analysis (see Appendix A, Fig. 3 within) and both strains demonstrated enhanced sensitivity to the lethal effects of IR. Clearly, the *spt4Δ* strain is also sensitive to the S phase specific DNA damaging agents MMS and HU. A role in DNA damage response would be predicted for any putative interactive target of BRCA1 given the many documented interactions of BRCA1 with other recombination or checkpoint proteins. This appears to be the case for *SPT4* as deletion of this gene leads to enhanced IR, MMS and HU sensitivity. Moreover, a physical interaction has been identified between endogenous yeast Spt4p and BRCA1 using co-immunoprecipitation (see below and Appendix A; Fig 5 within). The *spt5* truncation strain (*yml009w-bΔ*) also appears to be inhibited by HU and MMS but to a far lesser extent than the *spt4Δ* strain which correlates with its decreased ability to suppress BRCA1-induced lethality when compared to *spt4Δ* strain (Appendix A; Fig. 1 within). The shorter *spt5* truncation (which produces a longer Spt5p product) showed little or no inhibition following exposure to HU or MMS suggesting that the C terminal deletion defined by the overlapping *yml009w-bΔ* is important for resistance to DNA damaging agents. These results (sensitivity of the *spt4Δ* to IR, MMS and HU) were similar to that

seen for the *ccr4Δ* strain that was previously determined to be a member of the *RAD9*-dependent checkpoint repair pathway and also suppressed BRCA1-induced lethality in yeast.

As described in the introduction, defects in members of the CCR4 network have been predicted to result in sensitivity to agents such as hydrogen peroxide, which induce oxidative DNA damage mainly in the form of SSBs. We therefore exposed the diploid *CCR4* deletion mutant and WT to H_2O_2 and observed enhanced sensitivity of the *ccr4Δ* strain to this treatment when compared to WT (Fig. 2). Thus enhanced sensitivity of the

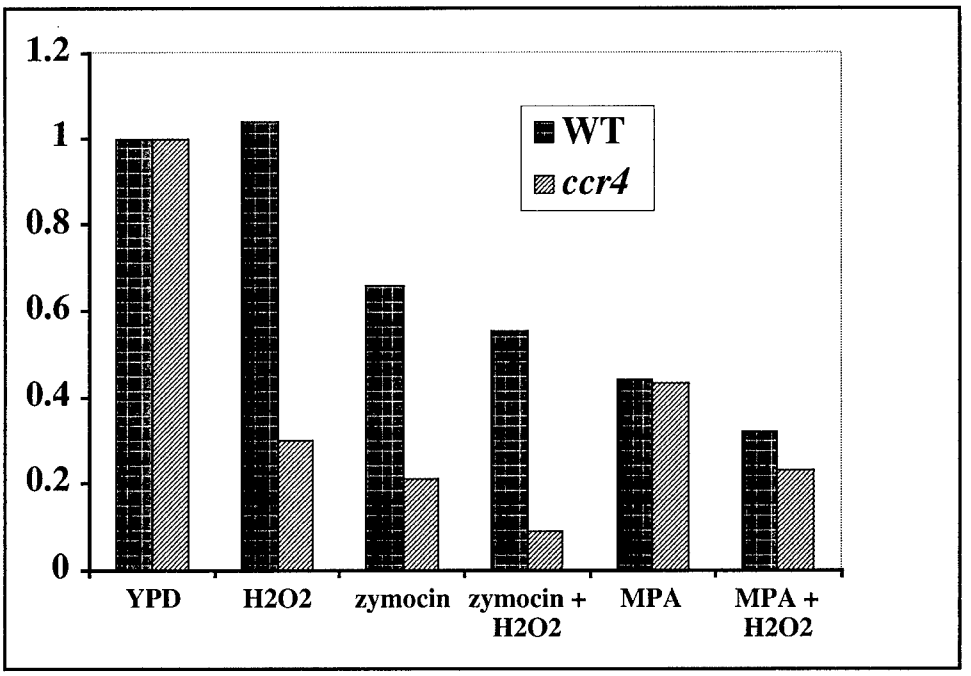

Fig. 2. *CCR4* mediates resistance to the lethal effects of hydrogen peroxide. Stationary WT and *ccr4Δ* diploid cells (in stationary G1 phase) were treated with zymocin, MPA or 6AU for 3 hours in liquid YPD prior to exposure to 0 or 25 mM H2O2 for 1 hr .in water. Following treatment, cells were immediately diluted in water and plated for survival (of colony forming ability) on YPD plates. H2O2 treatment alone is lethal in *ccr4Δ* cells but not WT. Pretreatment of cells to all transcription elongation inhibitors (including 6AU, data not shown) augmented the lethal effects of H2O2. Results are the average of 3 independent experiments.

ccr4Δ strain to H_2O_2 indicates that enhance sensitivity of this mutant to IR may result primarily from the induction of IR mediated SSB damage encountered by the elongating transcription apparatus. We also exposed WT and *ccr4Δ* cells to transcription inhibitors in the presence or absence of H_2O_2 to determine if these agents could synergistically enhance the lethality of oxidative DNA damage (Fig 2). As expected we observed enhanced lethality following exposure of the *ccr4Δ* strain to zymocin when compared to WT and this was augmented in the presence of H_2O_2. Interestingly, following exposure of the WT and *ccr4Δ* strain to mycophenolic acid (MPA), both strains demonstrated similar levels of lethality, which was further increased for both strains in the presence of H_2O_2. The different genetic requirements for MPA and zymocin induced lethality suggests that MPA and zymocin inhibit transcription elongation by different molecular mechanisms. Furthermore, while DNA damage induced by MPA in WT strains appears

to be poorly repaired, damage induced by zymocin in WT cells may be reversible and subject to recombinational repair. Thus *CCR4* appear to be required for resistance to oxidative damage and suggests that the CCR4 damage response network may be required for the repair of SSBs within actively transcribing genes.

c. Characterize the IR^S deletion mutants. Determine for IR^S deletion mutants whether IR sensitivity is the result of a defect in recombination or checkpoint functions as previously described. Some BRCA1 interacting genes may be members of the post replication repair pathway (*i.e.* similar to *RAD6* and *UBC13*). We will use epistasis analysis in yeast to identify what repair pathway newly identified candidate genes are members of. (Months 8-24).

We initially identified among mutants that suppress BRCA1-induced lethality, a striking overlap in sensitivity to the toxin zymocin secreted by the yeast *Kluyveromyces lactis* (see Table 2 within year 1 annual report). Sensitivity to zymocin was also found to overlap with most of the mutants that are sensitive to IR suggesting that zymocin may be inducing a spectrum of DNA damage similar to that produced by IR (36). The overlap between mutants that suppress BRCA1 induced lethality and those with sensitivity to zymocin suggested that BRCA1 and zymocin utilize similar molecular mechanisms to induce lethality. Furthermore, the cross sensitivity of these mutants to IR also suggests that BRCA1 and zymocin are inducing DNA damage in the form of DNA double strand breaks (DSBs) as previously suggested (37). Moreover, BRCA1 and zymocin appear to execute their lethal functions at the G1/S phase boundary since both have been described as eliciting a pronounced G1 arrest. Not surprisingly, in late G1 a large amount of transcriptional activity occurs in a cell cycle dependent manner to produce the gene products required for entry into S phase and the onset of replication. Thus similar to zymocin, BRCA1 may be inhibiting yeast transcription in late G1 by interfering with transcription elongation mediated in part by physical interactions with Spt4p and Spt5p. Therefore from these overlapping relationships several predictions can be made regarding the molecular activity of BRCA1 in yeast. Firstly, BRCA1-mediated inhibition of transcription would be predicted to result in DSB damage at actively transcribing regions including within the *GAL::BRCA1* fusion on the high copy, selectable (*URA3*) BRCA1 yeast expression plasmid. Furthermore, this activity would be predicted to be absent under conditions where there is no BRCA1-induced lethality (*i.e.* in the isogenic *spt4Δ* strain). We therefore examined whether BRCA1 induction in yeast resulted in plasmid degradation and loss in WT and *spt4Δ* diploid yeast cells.

We used both genetic and physical approaches to determine that plasmid loss occurred following BRCA1 induction in WT but not in *spt4Δ* cells (Fig. 3). Following galactose induced expression of BRCA1 in WT stationary (G1) cells, plasmid loss was observed both genetically by the loss of the *URA3* marker (Fig 3A) and physically by the loss of detectable plasmid DNA using Southern blotting (Fig. 3B). Under conditions where BRCA1 was not induced plasmid was retained both genetically and physically (Fig 3). Conversely, in the *spt4Δ* strain, the BRCA1 plasmid was maintained following BRCA1 expression in the presence of galactose. Furthermore, to confirm that BRCA1 was transcribed and translated in *spt4Δ* cells we determined using *in situ*

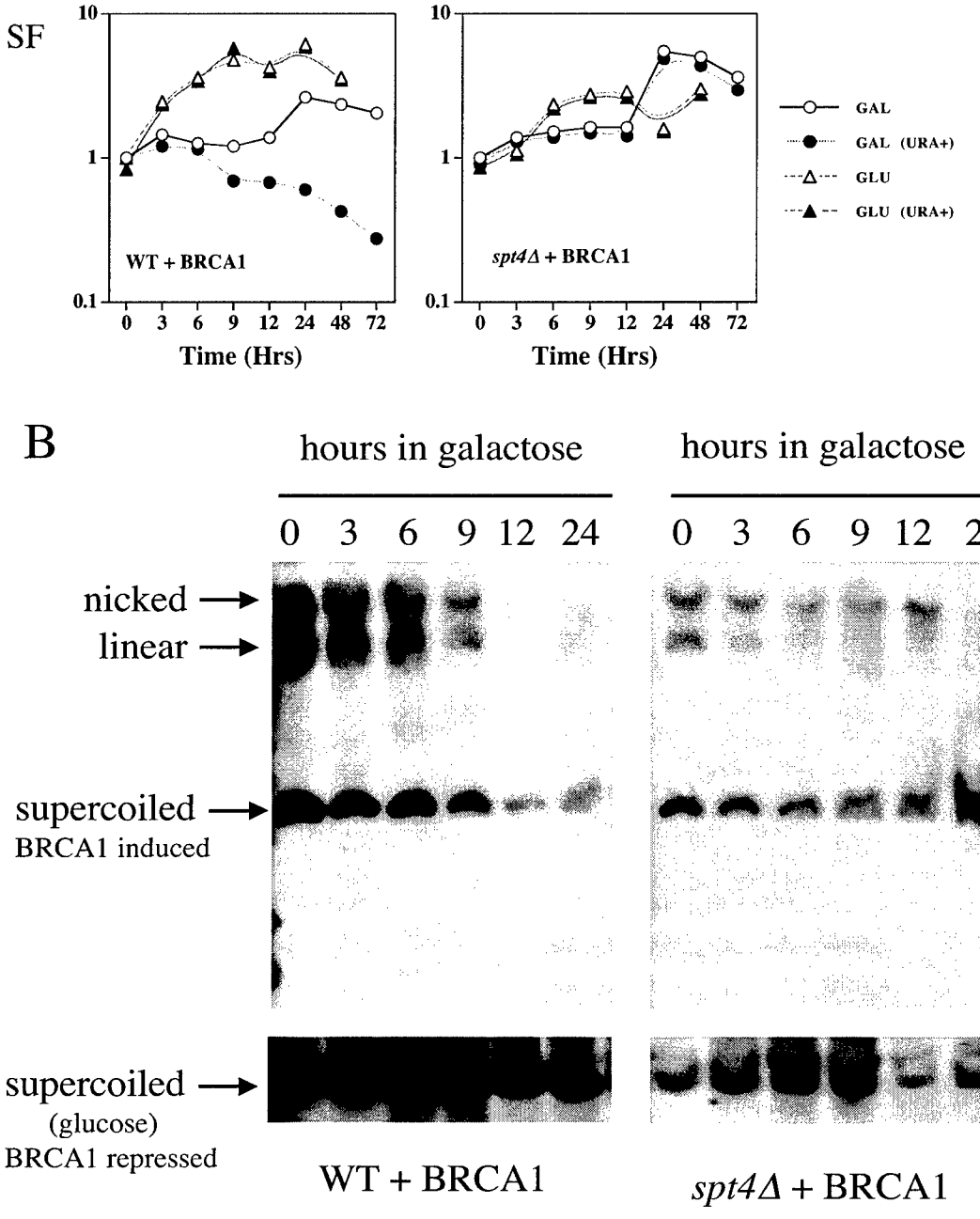

Fig. 3. Panel A: The high copy *GAL*::BRCA1 plasmid is maintained following BRCA1 expression in *spt4Δ* diploid yeast cells. WT and *spt4Δ* diploid yeast strains containing the high copy *GAL*::BRCA1 expression plasmid were grown to stationary phase (> 95% of cells were in G1 at t = 0 hr) in synthetic complete (SC) glucose medium lacking uracil (to repress BRCA1 expression and maintain plasmid selection). Cells were washed in water and placed in twice the volume of SC galactose medium lacking uracil to express BRCA1. Cells were also placed in SC glucose – uracil medium to serve as non-induced controls. .Cell viability was determined by plating aliquots at the indicated time points onto nonselective

rich medium (YPD) agar and allowing the cells to grow for 2 –3 days at 30°C. Plasmid loss among viable cells was determined by replica plating the resulting YPD colonies onto SC glucose – uracil to detect the presence or absence of the plasmid *URA3* marker (URA+). Initial (t = 0 hr) cell viability ranged from 0.42 – 1.1 x 10^7 cells/ml and increased to a maximum of 2.1 –4.1 x 10^7 cells/ml during the length of the time courses. Surviving fractions (SF) were determined by normalizing to the initial viable cell count for each growth condition. Colonies obtained following induction of BRCA1 in SC galactose – uracil have been indicated by circles (GAL). Colonies obtained following repression of BRCA1 in SC glucose – uracil medium have been depicted by triangles (GLU). A dramatic loss of the high copy (~60 copies per cell) plasmid marker can be seen only in the WT diploid strain following BRCA1 induction. Panel. B. Deletion of *SPT4* suppresses the loss of the high copy *GAL*::BRCA1 plasmid following galactose induced expression of BRCA1. Cell samples (3ml) from the above time course were collected by centrifugation and the supernatant fluid discarded. Pellets were frozen and genomic DNA phenol extracted as previously described (2). The *GAL*::BRCA1 plasmid probe was non-radioactively labeled using the CDP-STAR detection kit as per manufacturers instructions (Amersham). Aliquots of extracted DNA were separated on a 0.7% agarose gel and Southern blotted as previously described (6) (5). Expression of BRCA1 in a WT cell (*i.e.* those grown in galactose) results in a severe time dependent increase in plasmid loss. No plasmid was detectable after 48 or 72 hr growth in galactose (data not shown). Isogenic cells deleted for the transcription elongator *SPT4* maintain the plasmid following BRCA1 expression. Both strains maintain the *GAL*::BRCA1 plasmid when grown in SC glucose – uracil. Plasmid loss is not due to malsegregation as under these experimental conditions loss of the plasmid marker in galactose grown WT cells is greater than the amount of cell growth. (See Panel A). These results indicate that BRCA1 expression results in an elongation dependent plasmid loss in WT *Saccharomyces cerevisiae* cells. We expect that under these experimental conditions, elongation of the BRCA1 mRNA will be severely inhibited in the WT strain exposed to galactose while the *spt4Δ* strain will be less affected. Northern blots to demonstrate this are currently in progress.

immunofluorescence that BRCA1 protein was expressed at similar levels in both WT and *spt4Δ* cells (see Fig 4B in Appendix A). These results clearly show that BRCA1 expression results in plasmid breakage and loss even though the *GAL::BRCA1* plasmid is a high copy (2u based) vector. We are currently using northern analysis to examine whether galactose induction of BRCA1 protein results in transcript elongation defects for the *GAL::BRCA1* transcript. A further prediction is that exposure of yeast cells to BRCA1 or zymocin that results in DNA DSB damage will activate the cell cycle DNA damage dependent checkpoint system. Since a hallmark of S phase checkpoint activation by DNA damage is phosphorylation of Rad53p, we are currently using a yeast Rad53p-TAP tagged fusion strain and anti-TAP antibody to identify whether exposure to zymocin or BRCA1 expression can activate the DNA damage checkpoint response in this manner. In preliminary experiments (data not shown) zymocin appears to induce moderate phosphoryltion of Rad53p at 8 hours following toxin exposure. Such activation of Rad53p is a clear indirect indicator of the presence of DNA damage in S phase similar to that induced by MMS.

In related experiments, we have determined that a damage inducible (DIN) plasmid that has the promoter of *RNR3* fused to the β-galactosidase gene is not induced for lacZ expression in *ccr4Δ* or *spt4Δ* diploid strains exposed to the DNA damaging agent MMS while the *dhh1Δ* strain is hyperinduced for lacZ expression following identical MMS exposure (see Fig. 4A within Appendix A). We predict from our model that the *ccr4Δ* strain may suffer enhanced plasmid loss due to transcriptional elongation defects that occur when the induced LacZ gene encounters bulky MMS damage. To test this we therefore exposed WT and *ccr4Δ* cells containing the *DIN::*LacZ expression plasmid to

MMS for increasing amounts of time in liquid medium and determined the percentage of plasmid retention among the survivors plated to nonselective YPD plates (Table 1).

Table 1. Enhanced lethality and *DIN::*LacZ plasmid loss in *ccr4Δ* diploid cells exposed to methyl methanesulfonate (MMS)

Exposure Time (hours)*	S.Fraction WT + *DIN::*LacZ (0.03% MMS)	Plasmid Retention (%)**	S. Fraction *ccr4Δ* + *DIN::*LacZ (0.01% MMS)	Plasmid Retention (%)
0	1.0	96.1	1.0	96.2
3	0.83	88.2	0.84	81.9
6	0.53	95.1	0.29	75.3
9	0.37	92.2	0.077	56.8
12	0.18	90.0	0.0060	60.0

* Stationary cells (3 day cultures) in SC GLU-LEU liquid medium were diluted 1:1 in fresh medium and exposed to MMS with vigorous shaking at 30°C for the indicated times.

**% Plasmid retention was determined by the presence of the LEU2 *DIN::*LacZ plasmid marker . Presence or absence of the plasmid marker was determined by replica plating colonies surviving on nonselective YPD plates following MMS exposure to SC-LEU medium.

Results are the mean of two independent experiments

As expected, the *ccr4Δ* strain was more rapidly killed at a lower MMS dose (0.01% *vs* 0.03%) when compared to the WT strain. Furthermore, less plasmid was retained by the *ccr4Δ* strain as compared to WT. These results suggest that MMS damage may result in enhanced plasmid loss in the *ccr4Δ* strain due to transcriptional elongation arrest when actively transcribing genes (*i.e.* LacZ) encounter DNA damage. In preliminary results with the diploid *dhh1Δ* strain containing the *DIN::*LacZ plasmid and exposed to MMS, less lethality and more plasmid retention was observed as compared to the *ccr4Δ* strain. Thus the enhanced *DIN::*LacZ expression following exposure of *dhh1Δ* to MMS (see Appendix A, Fig 4A within) may be a function of less cellular lethality combined with longer plasmid retention time following MMS treatment.

In order to fully understand the molecular mechanism and genetic overlap in genes required for BRCA1 and zymocin-induced lethality, we screened the diploid deletion collection for strains that showed either enhanced resistance or enhanced sensitivity to the lethal effects of zymocin. Deletion strains that show enhanced resistance to zymocin are considered to be direct interactive toxin targets (TOT) required for the growth inhibitory and lethal effects of zymocin. Deletion strains that show enhanced sensitivity to the toxin are thought to represent defects in processes related to toxin degradation (*i.e.* enhance intracellular persistence of the toxin) or defects in the ability to "repair" the cellular damage induced by the toxin. A complex pattern of zymocin resistance emerged since 806 gene deletion strains (16.6% of the nonessential genome) demonstrated enhanced sensitivity to the lethal action of the toxin (see Appendix B). Another 119 deletions demonstrated increased resistance to the lethal

effects of zymocin of which, 13 completely abrogated both the lethal and growth suppressive effects of the toxin (See Appendix C).

Many of the zymocin sensitive mutants were identified as deletions that decreased cellular growth rates in the absence of zymocin (32%) or interfered with proteolytic processing, trafficking and/or protein degradation functions (16.4%). For example, 31 vacuolar protein sorting (VPS) mutants were identified and 171 mutants were identified that affected mitochondrial function including 37 mutants that were in mitochondria ribosomal protein subunits (MRP and MRPL). These results suggest that slowing the growth rate of yeast by deleting genes in processes necessary for rapid growth such as mitochondrial functions significantly enhanced sensitivity to the toxin. Presumably, slowing the growth rate may promote intracellular persistence of the zymocin toxin, which would enable it to evade rapid degradation.

As previously described (36), many of the zymocin sensitive mutants overlap with mutants identified in screens for IR sensitivity. In the zymocin screen (Appendix B and C), we identified a total of 103 zymocin sensitive mutants that were also found to be IR sensitive in our previous screens. These included a number of radiation resistance genes involved in recombination (*RAD6, RAD27, RAD50, RAD51, RAD52, RAD54, RAD61* and *XRS2*) as well as genes in G1 and S phase checkpoint response including *MRC1, RNR1, RNR4, HUR1, OCA1, PIN4, CLN3, FAR7* and *FAR10*. Interestingly, a number of genes that have been implicated in mitochondrial DNA recombination, meiotic recombination or repair of DNA damage were identified including *ABF2, APM1, CHL1, FYV6, HEX3, HFM1, IXR1, MHR1, MSC2, MSH1, PIF1, SOH1* and *TPP1*. Intriguingly, *MHR1* is required for the recombinational repair of oxidative damage in mitochondrial DNA (21) and has been found to interact with the RNA pol II holoenzyme complex (9) suggesting *MHR1* may play a critical role in the repair of zymocin arrested transcription complexes. Furthermore, similar to *MHR1* many genes involved in the stress response (i.e. respond to oxidative damage or stress) were found to be zymocin sensitive when deleted. These include *CCW14, DBF2, GON7, GPX2, GRE3, GSH2, MGM101, OCA1, LIP2, LIP5, LRE1, PHO85, PIL1, RPN4, RVS161, RVS167, SLM2, SRF1, SRF4* and *WHI2*. A large number of transcriptional cofactors that interact with the RNA pol II holoenzyme complex were also found including many key members of the CCR4 damage response network. A number of zymocin sensitive deletions were found to also confer resistance or sensitivity to either the K1 killer toxin (*CWH41, FYV4, FYV6, FYV8, FYV10, KRE1* and *KRE2*) or the toxin secreted by *Pichia farinosa* (*PKR1* and *SPF1*). This suggests that these toxins share common determinants in the genetic pathways that mediate lethality, which in the case of the related *Pichia* species *P. acaciae* involves checkpoint arrest and DNA damage (18) (17).

Among the 13 deletion strains that were found to be completely resistant to zymocin, 10 had been previously identified (*ATS1, CHS3, CHS7, ELP2, ELP3, ELP6, KTI11, KTI12* and *SKT5*) and four (*TRM9, IPT1, YDL041W* and *YDR417C*) represent new TOT mutant strains. Interestingly, *TRM9, YDL041W* and *YDR417C* were previously found to be sensitive to IR as were a number of other zymocin resistant mutants (*ADO1, BUD19, LOC1, MRT4, NAT1, NOT3, RAI1, RLR1, RSA1* and *YML036W*). Furthermore, deletions of either *MRT4* or *RAI1* were found to exhibit checkpoint defects following IR. Therefore, similar to *CCR4*, these two IR resistance genes may play a critical overlapping role in the cellular checkpoint pathways that respond to zymocin and IR. Not

surprisingly, Mrt4p and Rai1p as well as a number of tRNA methyltransferases (similar to Trm9p) have been found to bind to phosphorylated synthetic peptides that mimic the C terminal domain (CTD) of RNA pol II (31). Since phosphorylation of the CTD domain of RNA pol II regulates transcription elongation (30) and the G1/S damage response protein Hrr25p (13) also binds the phosphorylated CTD of RNA pol II (31), many of the zymocin resistant mutants including *mrt4Δ*, *rai1Δ* and *trm9Δ* may be defective in a G1/S damage checkpoint transition function that utilizes RNA pol II to sense DNA damage in actively transcribed genes. Thus the identification of these zymocin sensitive and resistant deletion mutants is supportive of the molecular model for the involvement of RNA pol II and transcriptional elongation as key components in the mechanism of BRCA1-induced lethality.

Task 2. For yeast genes with human orthologs, we will determine if physical protein-protein interactions occur between the newly identified proteins and BRCA1 in yeast and human cells. (Months 13-30)

a. Design and construct a series of plasmids for yeast two hybrid analysis using BRCA1 as the "bait" and candidate yeast (or human orthologs) identified in Task 1 as the "prey". (Months 8-30).

As discussed in the year 1 annual report, we have used the Gateway cloning system (Invitrogen) to construct bait and prey vectors (in the yeast expression plasmids pDEST22 and pDEST32) to determine the interaction of Dhh1p and DDX6 with the C terminal BRCT domain of BRCA1 and the N terminal RING domain of BRCA1. These two domains have been described as conferring a majority of the protein-protein interactions described for the BRCA1 molecule. We failed to see any significant two-hybrid interaction among these proteins, however we were able to successfully co-immunoprecipitate BRCA1 and Dhh1p in yeast cells as well as co-immunoprecipitate DDX6 and BRCA1 proteins when expressed in MCF7 cells (*i.e.* see Fig 5 within year 1 annual report). We have continued using the Gateway system to examine whether two-hybrid interactions in yeast can be observed using as bait (or prey) our genetic target (*SPT4*) found to suppress BRCA1-induced lethality to the greatest extent in yeast and its human ortholog hSPT4. Again, we found no significant two-hybrid interaction between yeast Spt4p or hSpt4p and either the BRCT domain or the RING domain found within the BRCA1 tumor suppressor protein (Fig. 4). We have however, detected *trans*-activation of transcription at the *HIS3* locus (Fig. 4B) when the BRCT domain was fused to the DNA binding domain of *GAL4* (in pDEST32 but not pDEST22) as has been previously reported (15). This indicates that the BRCT domain is biologically active in our host yeast strain (MaV203). However, similar to Dhh1p, we have detected physical interaction of yeast Spt4p with BRCA1 using co-IP (see Appendix A Fig 5 within) even though interaction was not detected using two-hybrid analysis. Currently we are transiently co-expressing BRCA1 and hSpt4p in the human breast cancer cell line MCF7 to determine if hSpt4p interacts with BRCA1 in a manner similar to DDX6. These results suggest that BRCA1 may be interacting with Spt4p, Dhh1p and our other transcription factors that suppress BRCA1-induced lethality as part of a large RNA pol II holoenzyme elongation complex

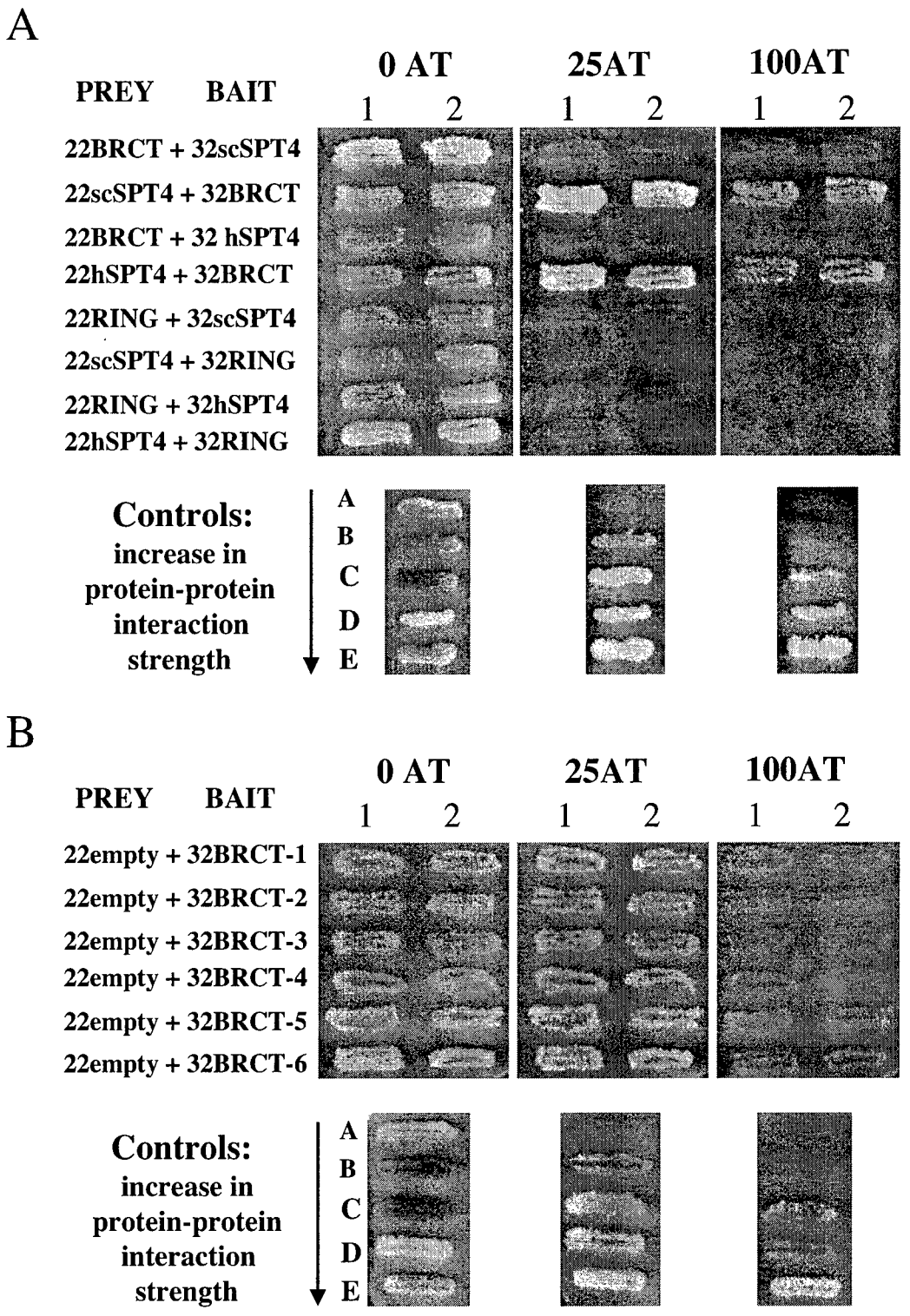

Fig. 4. Yeast and human Spt4p do not directly interact with the BRCT or RING domains within BRCA1 using two-hybrid analysis. Panel A: The yeast two-hybrid host strain MaV203 containing the GAL1 UAS upstream of *HIS3* was transformed with "bait" plasmid (pDEST32) containing either yeast

SPT4 (32scSPT4), human SPT4 (32hSPT4), BRCA1 BRCT domain (32BRCT) or BRCA1 RING domain (32RING) plus the appropriate "prey" plasmid (pDEST22) containing either yeast SPT4 (22scSPT4), human SPT4 (22hSPT4), BRCA1 BRCT domain (22BRCT) or BRCA1 RING domain (22RING) interaction targets. Two individual transformed strain isolates (1 and 2) were patched onto synthetic complete (SC) glucose –leucine-tryptophan (GLU-LEU-TRP) and allowed to grow for 24 hours. These patched transformants were subsequently replica plated to SC-LEU-TRP-histidine plates that contained various concentrations (0, 10, 25, 50, 75 and 100 mM) of the histidine inhibitor 3-Amino-1,2,4-Triazole (AT). Control MaV203 strains (A, B, C, D and E) contain bait and prey plasmids and show increasing strength of the two-hybrid protein-protein interactions. Control A: pPC97-empty + pPC86-empty, no interaction; Control B: pCB97-humanRB + pPC86-humanE2F1, weak interaction; Control C: pPC97-DrosophilaDP + pPC86-Drosophila E2F, moderately strong interaction; Control D: pPC97-rat cFos + pPC86-mouse cJun, strong interaction; Control E: pCL1 encoding full length GAL4 + pPC86-empty, very strong interaction. Panel B: Strain MaV203 was transformed with six separate pDEST32 vectors expressing the BRCT domain (32BRCT-1 to 6) and pEXP-AD502, which is the pDEST22 plasmid with no insert (22empty). The BRCT domain fused to the GAL4 DNA binding domain demonstrates trans activation of the *HIS3* reporter gene. Plating conditions and controls are the same as described in panel A.

Alternatively, Dhh1p and Spt4p may require the full length (or internal regions) of BRCA1 to promote physical interactions. As described in the year 1 report, it has been impossible to clone full length BRCA1 into a Gateway entry clone using site-directed recombination (BP reactions). We have therefore placed *Not*I and *Sal*I restriction sites on the end of BRCA1 using the appropriately designed primers and PCR amplification. Following restriction digestion, we are using conventional "sticky end" ligation techniques to directionally clone full length BRCA1 into the Gateway donor plasmid pENTR1A. Once established and sequenced to confirm integrity, we will subsequently transfer the stable BRCA1 insert into pDEST22 and pDEST32 for rapid 2-hybrid analysis with Dhh1p, Spt4p, DDX6 and hSpt4p. We have also cloned the yeast genes *CCR4* and *YAF9* into the entry vector pDONR221 and are confirming gene integrity by sequencing. Once confirmed, these donor vectors will be used to rapidly shuttle *CCR4* and *YAF9* into pDEST22 and pDEST32 for two-hybrid analysis with full-length BRCA1 and the BRCT and RING domains within BRCA1.

b. For candidate genes that show physical interaction in yeast, design and construct a series of plasmids for mammalian two hybrid analysis using BRCA1 as the "bait" and candidate human genes as the "prey". (Months 12-36).

Given our inability to detect two-hybrid interactions in yeast for DDX6 or hSpt4 with the BRCT or RING domains from BRCA1, we have chosen not to construct the corresponding mammalian two-hybrid vectors since observing two-hybrid interactions in mammalian cells but not in yeast is highly unlikely. For cloned targets currently under investigation (*i.e. YAF9* and *CCR4*) mammalian two-hybrid interactions in mammalian cells will be performed only if two-hybrid interactions can be detected in yeast.

c. Confirm by co immunoprecipitation techniques the occurrence of physical interaction of BRCA1 protein with candidate human proteins that were previously shown positive for two hybrid interactions in mammalian cells. (Months 16-36).

With the finding that both Dhh1p (see Fig. 5 within year 1 annual report) and Spt4p (Appendix A, see Fig 5 within) interact with BRCA1 using co-immunoprecipitation but not with two-hybrid analysis in yeast, we are presently examining protein-protein interactions in yeast using a variety of yeast resources that are now cloned within the lab or commercially available. Currently, we are co-expressing a C terminal FLAG tagged hSpt4p (human Spt4 protein) and BRCA1 (from the *GAL::BRCA1* plasmid) in *spt4Δ* diploid yeast to identify whether hSpt4 physically interacts with BRCA1 in yeast. Anti-FLAG antibody will be used to co-IP BRCA1, while anti-BRCA1 antibody (AB-1; Calbiochem) will be used to co-IP hSpt4p. We have also cloned hSpt4 into the human expression vector pDEST40. We are also transiently, co-expressing hSpt4p and BRCA1 in MCF7 cells and using co-IP analysis to determine if BRCA1 and hSpt4p physically interact in human cells.

Yeast strains and/or plasmid collections are also available where individual "tagged" proteins can be either overexpressed (25) or alternatively, expressed from their natural promoter (12). These collections utilize either a C terminal TAP tag or an N terminal GST tag fused to the protein of interest. In preliminary experiments we have used the TAP tag collection (12), which utilizes expression from the endogenous promoters to show that yeast Ccr4p, Spt5p and Rap1p may physically interact with BRCA1 in yeast (data not shown). Rap1p was chosen as a putative interactive target since transcriptionaly active genes (in particular GAL genes) have been localized to the nuclear periphery at the nuclear pore (8) suggesting a molecular mechanism for the rescue of BRCA1-induced lethality by a variety of nuclear pore deletion mutants (Fig. 1). However, to maximize detection of proteins that interact with BRCA1, we are using the Exclone collection (maintained here at Duke as an open accessible resource; see www.dbsr.duke.edu/yeast/Genome%20Libraries/exclones.htm) developed to maximize expression of individual yeast genes. There are a total of 6080 individual yeast strains arrayed in 96-well plates, each containing a plasmid expressing a different full-length yeast open reading frame (ORF) fused to glutathione S-transferase (GST). The GST-fusion expression is under the control of the *CUP1* promoter and the plasmid copy number can be increased by growth in synthetic complete medium lacking leucine. Thus protein expression can be maximized by enhancing plasmid copy number followed by induction of the *CUP1* promoter in copper sulfate (CuSO4). Since the GST-fusion plasmid also contains a *URA3* marker, we have used PCR mediated gene transplacement to replace the *URA3* marker on the *GAL::BRCA1* plasmid with *HIS3*. This will allow co-selection of the appropriate GST fusion plasmid (containing *DHH1, CCR4, SPT4, YAF9* and others) with the *GAL::BRCA1* plasmid. Cells containing both plasmids will be induced in galactose medium lacking leucine and containing CuSO4 such that both BRCA1 and the GST tagged protein of interest will be expressed at high levels. Therefore, we will subsequently use anti-BRCA1 antibody and glutathione-Sepharose beads to rapidly perform co-immunoprecipitation and GST "pull down" experiments without the need for further plasmid construction and sequence confirmation which can be very time consuming even with the versatile Gateway cloning system (Invitrogen).

Task 3. Determine if newly identified BRCA1-interacting genes contribute to genomic stability following IR damage in human cells. (Months 13-36).

a. Using siRNA technology, down regulate the expression of BRCA1 in normal human fibroblast cells and determine if cells are sensitized to the killing effects of IR. (Months 13-18).

We have used siRNA transfection techniques to successfully down regulate BRCA1 expression in MCF7 human breast cancer cells that express BRCA1 (Fig. 5) as well as immortalized normal (DU99) human cells (data not shown). MCF7 cells were

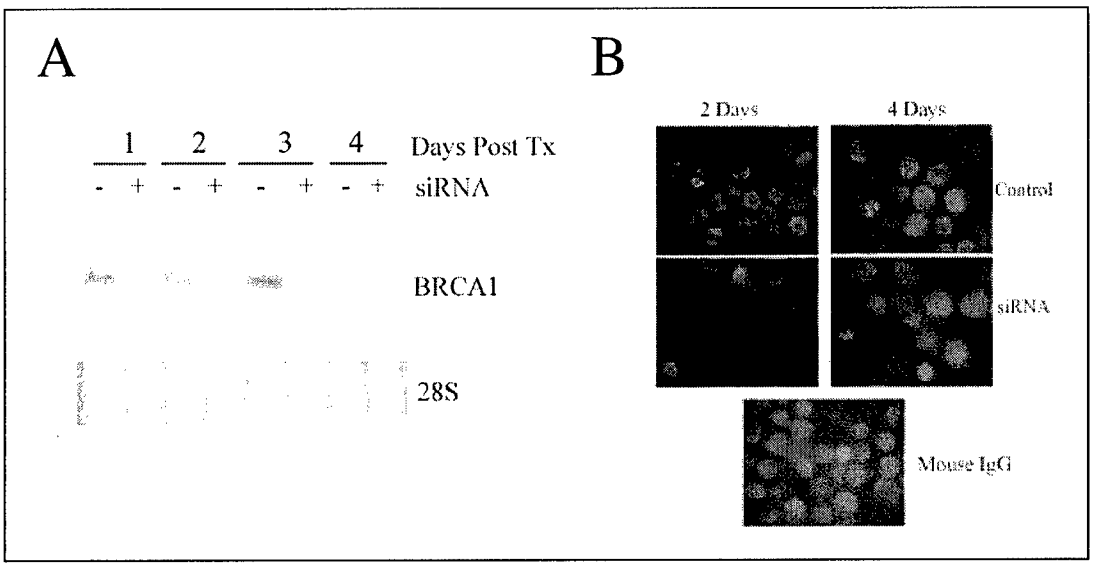

Fig. 5. siRNA-mediated down regulation of BRCA1 expression in MCF7 cells. Cells were either transiently transfected with BRCA1 specific siRNA designed for the down regulation of BRCA1 mRNA or nonsense siRNA to serve as a negative control. Panel A: At the indicated times RNA was extracted from the transiently transfected MCF7 cells, and equivalent amounts were separated by agarose gel electrophoresis. BRCA1 message was visualized using randomly labeled BRCA1 exon 11 randomly labeled with ^{32}P. Ribosomal 28S RNA serves as a loading control. Panel B: Logarithmically growing MCF7 cells were exposed to siRNA specific for BRCA1 or nonsense control siRNA for the indicated times, fixed onto a slide and incubated with anti BRCA1 mouse monoclonal antibody (AB-1). The location of anti-BRCA1 antibody binding was detected with an anti-mouse secondary antibody conjugated with Alexa fluor 488. No BRCA1 signal was observed in the nuclei of siRNA cells or in cells treated with non immune mouse IgG. Nuclear localized BRCA1 some containing foci are abundant in MCF7 cells treated with control siRNA 2 or 4 days following transfection

either transiently transfected with siRNA designed for the down regulation of BRCA1 mRNA or nonsense siRNA as a negative control. RNA was extracted from MCF7 cells at 1, 2, 3 and 4 days post transfection, separated by agarose gel electrophoresis and BRCA1 message was visualized using randomly labeled probe from exon 11 of BRCA1 (Fig. 5A). BRCA1 message was effectively down regulated by siRNA at all time points following transient transfection. Furthermore, severely reduced levels of BRCA1 protein were observed following indirect immunofluoresence of siRNA transfected MCF7 cells at either 2 or 4 days post transfection (Fig. 5B). Since BRCA1 is required for homologous recombination (28), we have utilized a recombination assay that detects an altered fluorescent "signal" from a recombined green fluorescent protein (GFP) substrate using FACS cell sorting procedures (33). Unrecombined substrates fluoresce blue whereas recombined substrates fluoresce green such that the ratio can be used as a direct measure of recombination proficiency. This recombination assay is particularly relevant since it can detect defects associated with loss of BRCA1 function in the absence of DNA damage (33). Thus this assay is particularly useful for assessing whether newly identified

genetic determinants affect genomic integrity within the BRCA1 pathway. We therefore transfected MCF7 cells with siRNA specific for BRCA1 as well as control siRNA and 24 hours later we transiently transfected the recombination substrate (in direct orientation). FACS cell sorting was performed 48 hours later and fluorescent green/blue ratios determined. Using this approach we detected a 4.2 fold increase in recombination (i.e. 0.28 *vs.* 0.67 green/blue ratio, mean of three experiments) in the control vs. the cells treated with siRNA specific for BRCA1 mRNA interference. Thus this approach will be able to successfully detect decreased recombination frequencies associated with defective genes within the BRCA1 signaling pathway.

b. Using siRNA technology down regulate highly conserved, candidate BRCA1-interacting genes in normal human fibroblasts to demonstrate sensitivity to the killing effects of IR. (Months 16-36).

We have obtained siRNA specific for the ortholog of *DHH1* (DDX6) and experiments to down regulate this gene in MCF7 and DU99 cells are in progress. In preliminary experiments, we have determined that overexpression of DDX6 can modulate the ionizing radiation sensitivity of transiently transfected MCF7 cells (data not shown). As described above for BRCA1 (Fig 5), we are down regulating DDX6 in MCF7 and DU99 to determine if the spontaneous recombination frequencies declines following transient transfection of the recombination substrates described above.

Key Research Accomplishments:

- Identification of transcription elongation as the major target for BRCA1-induced toxicity in yeast.
- Development of a molecular model for BRCA1-induced lethality that incorporates zymocin toxicity and ionizing radiation sensitivity of mutants that suppress BRCA1-induced lethality.
- Identification of components of the nuclear pore as a major interactive target required for BRCA1-induced lethality in yeast.
- Development of an *in situ* indirect immunofluorescence technique for the detection and localization of BRCA1 protein expression within fixed yeast.
- Detection of degradative DNA damage (i.e. plasmid loss) using physical (Southern blotting) and genetic assays.
- Determination that *ccr4Δ* mutants are hypersensitive to oxidative damage which, is augmented by transcription inhibitors including zymocin, mycophenolic acid and 6-azauracil.
- Determination that loss of the DNA damage inducible *DIN*::LacZ plasmid is enhanced in *ccr4Δ* cells as compared to WT following exposure to MMS.
- Identification of yeast Spt4p as a genetic and physical interactive partner of BRCA1 in yeast.
- Determination that yeast *SPT4* and *SPT5* confer resistance to IR and the S phase specific DNA damaging agents hydroxyurea and methyl methanesulfonate.

- Establishment by two-hybrid analysis that yeast and human Spt4p does not directly physically interact with BRCA1 in yeast.
- Confirmation that the BRCT domain within BRCA1 can trans-activate transcription when fused to the GAL4 DNA binding domain in pDEST32.
- Completion of genome-wide screen in diploid yeast to identify 806 zymocin sensitive and 119 zymocin resistant deletion mutants.
- Genetic and physical confirmation that DNA damage is a major component of zymocin induced lethality in yeast
- Cloning of *CCR4*, *YAF9* and human *SPT4* into Gateway compatible entry vector (pDONR221) for shuttling into two-hybrid expression

Reportable Outcomes: Paper in review for publication in Annals of Surgical Oncology

Abstract submitted and accepted for oral presentation at the Era of Hope DOD Breast Cancer Research Program's Meeting in Philadelphia, Pennsylvania, June 8–11, 2005.

BRCA1 INTERACTS WITH HIGHLY CONSERVED COMPONENTS OF THE TRANSCRIPTION ELONGATOR COMPLEX
T. J. Westmoreland, A. L. Selim, W. Y. Saito, G. Huper, J. A. Olson, J. R. Marks, and C. B. Bennett, Ph.D.

Conclusions:

This project has used the yeast *Saccharomyces cerevisiae* as a model system to identify new genetic and/or physical interactive targets of BRCA1. From the identification of these targets we appear to have elucidated the molecular mechanism of BRCA1 induced lethality in yeast. From an unselected pool, we identified deletion mutants that suppressed the G1 arrest and lethality induced by heterologous expression of the human tumor suppressor protein BRCA1. From this screen and overlapping sensitivity of BRCA1-interactive mutants to IR and zymocin we independently identified members of the CCR4 damage response network. The majority of these conserved genes including *CCR4, DHH1, DEF1, SPT4, YAF9, HCM1, YAP3, SUB1* and numerous nuclear pore components are required for transcription and confer resistance to both IR and transcription elongation inhibitors. Furthermore, these genes mediate a rapid G1 and S phase cell cycle progression following DNA damage. We *hypothesize* that conserved members of this network directly interact with BRCA1 in human cells to promote damage signaling from stalled RNA pol II transcripts blocked by IR-induced DNA single strand breaks during the mRNA elongation step. In *CCR4* deletion mutants these blocked transcription complexes are poorly repaired and elicit a prolonged, IR-induced checkpoint arrest at G1. Eventually, *ccr4Δ* cells adapt to this persistent damage and enter S phase where these stalled RNA pol II complexes serve as DNA replication blocks that are processed into lethal DSB damage. We hypothesize that interactions within this mRNA damage checkpoint response are highly conserved and can be used to identify orthologous protein interactions within human cells. Moreover, BRCA1 through physical interaction with Dhh1p, Ccr4p, Spt4p and other members of this mRNA damage response network, plays a critical role in the damage signaling and/or repair of RNA pol II

transcript complexes stalled by DNA lesions. Therefore defects in the human orthologues of these genes such as DDX6 or human SPT4 are predicted to play a key role in the maintenance of genomic stability. Genomic instability resulting from a loss of function in these genes may therefore contribute to the molecular initiation of breast cancer.

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Appendices:

A. Manuscript submitted to Annals of Surgical Oncology
B. Appendix Table: Zymocin sensitive diploid deletion mutants in *Saccharomyces cerevisiae.*
C. Appendix Table: Zymocin resistant diploid deletion mutants in *Saccharomyces cerevisiae.*

D. Abstract submitted and accepted for oral presentation at the Era of Hope DOD Breast Cancer Research Program's Meeting in Philadelphia, Pennsylvania, June 8–11, 2005.

Spt4 confers resistance to DNA damage and interacts with BRCA1 in yeast.

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Abstract

Background: Deletion of *SPT4* in the yeast *Saccharomyces cerevisiae* results in rescue of BRCA1-induced lethality following heterologous expression. BRCA1 interacts with RNA polII as does the human ortholog of *SPT4* which acts in a multisubunit transcription elongation complex to regulate transcription. We therefore examined whether yeast Spt4p physically interacts with the tumor suppressor BRCA1. **Methods:** We expressed BRCA1 in WT and *spt4Δ* diploid yeast cells from an inducible high-copy expression plasmid. Survival assays were performed in the *spt4Δ* strain following BRCA1 expression or exposure to various DNA damaging agents. The cell cycle progression of single (G1) WT and *spt4Δ* cells expressing BRCA1 were monitored microscopically. Co-immunoprecipitation (co-IP) using anti-Spt4p antibody was performed, and Western blotting confirmed interaction. Immunofluorescence (IF) of BRCA1 in WT and *spt4Δ* strains was used to confirm BRCA1 protein expression. **Results:** Deletion of *SPT4* completely suppressed the G1 arrest and lethality observed following heterologous expression of full-length BRCA1 in WT cells. Furthermore, Spt4p and BRCA1p physically interact as indicated by co-IP of BRCA1p with anti-Spt4 antibody from lysates of yeast cells induced for BRCA1 expression. IF of fixed yeast revealed BRCA1 expression in *spt4Δ* cells. Moreover, deletion of *SPT4* or C terminal truncation of its interactive partner *SPT5* resulted in sensitivity to ionizing radiation. **Conclusions:** BRCA1-induced lethality in WT yeast is abrogated by deletion of *SPT4*. Since the conserved Spt4p of yeast physically interacts with BRCA1, human Spt4 may similarly interact with BRCA1 in human cells to maintain genomic stability and suppress the onset of breast cancer.

Synopsis: Using yeast as a model system for the rapid identification of putative new breast cancer gene targets, we identified the conserved protein Spt4p as an interactive target of the tumor suppressor BRCA1. Consistent with the proposed role of BRCA1 in DNA repair, Spt4 was found to confer resistance to a variety of DNA damaging agents including ionizing radiation. Thus human Spt4 may similarly interact with BRCA1 to maintain genomic integrity following DNA damage.

Introduction

In hereditary breast cancer, changes in DNA repair ability have been identified that result from alterations in the BRCA1 and BRCA2 gene products. These genes have been found to be pleiotropic tumor suppressors that impact on recombinational repair of DSB damage, cell cycle checkpoint arrest following DNA damage, transcriptional regulatory mechanisms, and centrosome duplication [1 2 3 4]. Although most breast cancers are susceptible to treatment with ionizing radiation (IR) which induces double-strand break (DSB) damage, <10% of breast cancer cases correlate with alterations in BRCA1 or BRCA2 function [5]. This suggests that additional unidentified gene products may be altered in their expression or function resulting in the molecular changes responsible for disease progression and susceptibility to IR-induced lethality. Interactions with many other proteins involved in DNA repair have been described for BRCA1 and BRCA2 including those involving the recombination repair protein hRad51 [6], the DEAD/H box repair helicase BACH1, [7] and BARD1, [8] a gene thought to be involved in post replication repair of DNA damage. Therefore, it is probable that the pleiotropic phenotypes associated with BRCA1 mutations may result from aberrant interactions with one or more factors in many complexes. Alternatively, BRCA1 has further been proposed to form a large damage

surveillance complex that includes other tumor suppressor genes and repair proteins, such as MSH2, MSH6, MLH1, ATM, BLM [9] as well as the RAD50/MRE11/NBS1 repair complex [10]. This large complex in which BRCA1 appears to perform a scaffolding function has been hypothesized to guard against the loss of genome integrity by recognizing DNA damage and elicit a signal to initiate repair. Therefore, in human cells, BRCA1 has been proposed to maintain genomic stability through its function as a DNA damage recognition and signaling protein that binds to branched DNA structures [11 12]. It is also known to bind a number of other DNA repair associated proteins that includes BACH1, hRad51, BRCA2 and BARD1 [7 13 14]. Thus, the scaffolding functions of BRCA1 are thought to also coordinate the recombinational repair of DSBs.

BRCA1 is also involved in multiple transcription related functions including the regulation of p21, p53, Mdm2 and Gadd45 [15 1 16]. Furthermore, BRCA1 is known to bind the RNA pol II holoenzyme complex [6 17]. It has been suggested that BRCA1 may be serving as a key component in a critical genome surveillance mechanism by associating with hyperphosphorylated forms of RNA pol II [18] that can signal the presence of DNA damage when elongating RNA pol II complexes encounter DNA lesions (reviewed in [19 20]). Furthermore, BRCA1 can modulate phosphorylation of the RNA pol II carboxy-terminal domain (CTD) which appears to play a critical role in transcriptional elongation [21]. Interestingly, immunoprecipitation of BRCA1 co-purifies with active forms of RNA pol II and TFIIH [17] which itself plays multiple roles in DNA repair [22]. Recently, BRCA1 has been found to bind through one of the BRCT domains to a novel *co* factor of *BRCA*1 (COBRA1), which in turn binds to a specific genomic

site to promote chromatin decondensation [23]. COBRA1 has also been found as an integral component of the human *n*egative *el*ongation *f*actor (NELF) that binds to *DRB* (5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole) *s*ensitivity *i*nducing *f*actor (DSIF) to cause transcriptional pausing of RNA pol II holoenzyme complexes [24]. Not surprisingly, DSIF has been found to be required for transcriptional elongation by RNA pol II and is composed of the highly conserved human orthologs of the yeast Spt4 and Spt5 proteins [25].

Saccharomyces cerevisiae has served as the preeminent model organism for the identification of the genetic controls associated with DNA repair and checkpoint functions. This organism has also served as an important model organism for the elucidation of complex human systems since it shares a high level of genetic and functional homology with humans [26]. In fact most of the gene products involved in the repair of DSBs in humans were first identified in yeast. Heterologous expression of BRCA1 causes slow growth and lethality in both haploid [27] and diploid [28] WT yeast. Furthermore, heterologous expression of the C terminal BRCT domains of BRCA1 can stimulate transcription in yeast [29]. Mutations within the two tandem C terminal BRCT domain repeats of BRCA1 that have been associated with breast cancer disease abrogate this slow growth phenotype [27]. Moreover, the BRCT domain appears to be a common feature of proteins involved in checkpoint and DNA repair related functions [30 31] and mediate protein-protein interactions in yeast [32] and human [33] cells. Polymorphisms not associated with breast cancer do not interfere with the slow growth phenotype in yeast, suggesting that the biological effects in yeast accurately reflect biologically relevant molecular functions in human cells.

We previously described a prolonged G1 arrest and lethality that occurs following heterologous expression of BRCA1 in diploid yeast [28]. Using yeast as a model system to identify new BRCA1 interactive gene targets, we found in a screen of isogenic IR sensitive deletion mutants [34] that nullizygous deletions of the IR resistance genes *CCR4* or *DHH1* suppressed BRCA1-induced G1 arrest and lethality [28]. In this report we have identified *spt4Δ* as a potent genetic suppressor of BRCA1-induced G1 arrest and lethality in yeast. This null mutant was identified from a screen of a random pool of genetically tagged, isogenic diploid deletion mutant yeast strains that suppressed the slow growth phenotype normally observed following transformation of a high-copy *GAL*::BRCA1 plasmid and subsequent BRCA1 expression on galactose containing selective medium. Carboxy (C) terminal truncations of the cognate Spt5 protein also suppressed BRCA1-induced lethality suggesting that the C terminal domain of Spt5p may be required for physical interactions with Spt4p. The *SPT4* deletion was also found to result in enhanced sensitivity to a variety of DNA damaging agents known to promote genetic instability through the induction of DSBs. Furthermore Spt4p was found to physically interact with BRCA1 in yeast using co-immunoprecipitation (co-IP). Since Spt4 is highly conserved in human cells, these results suggest that BRCA1 may similarly interact with human Spt4 (hsSPT4) to maintain genomic integrity following DNA damage in human cells.

Methods

Genome-wide screen for the suppression of BRCA1-induced growth arrest. An isogenic pool of genetically tagged yeast diploid deletion strains containing 4653 individual nonessential

deletions was obtained from ResGen (Invitrogen). Yeast deletion strains were thawed from a frozen aliquot of the pooled deletion strains (200 ul, at room temperature) and grown by inoculating the thawed cells into a 50 ml volume of YPD (*y*east extract *p*eptone *d*extrose) liquid medium. Cells were maintained at 30°C with shaking until a cell count of 1-2 x 10^7 cells/ml was obtained. Cells were made competent as previously described [28] and 1-2 ug of the selectable (*URA3*) high copy *GAL*::BRCA1 expression plasmid was introduced into the pool of yeast deletion strains by transformation. This plasmid has full-length BRCA1 fused to the *GAL1* promoter [28]. When cells are grown in glucose, BRCA1 is highly repressed and cells containing the plasmid are viable; however, upon transfer to galactose containing medium, BRCA1 is expressed in large quantities resulting in slow growth and lethality. Following transformation, cells were plated directly to synthetic complete (SC) galactose containing medium (2%) lacking uracil (GAL-URA). Rapidly growing colonies were picked following 2 or 3 days incubation at 30°C. Individual colonies were streak purified by plating again to GAL-URA plates. Individual colonies were tested for the ability to suppress BRCA1-induced lethality by growing wild type (WT) and mutant strains containing the *GAL*::BRCA1 plasmid in liquid SC glucose containing medium (2%) lacking uracil (GLU-URA) until stationary phase was reached in 96 well dishes. Stationary phase cultures were serially diluted (5 fold) in sterile water and 2ul aliquots were replica plated to GLU-URA and GAL-URA solid medium using a 48 pin replica plating device. Isolates that showed enhanced survival on GAL-URA plates when compared to WT were processed for identification using colony PCR amplification and sequencing of the unique 20 bp tag associated with the deletion strain [35].

Identification of deletion strains by PCR amplification and sequencing of unique 20 bp tags. Cells from colonies were lysed in lyticase and the released genomic DNA was used as a template to PCR amplify the unique 20 bp tag that was incorporated into each deletion strain during strain construction. The PCR primers used for amplification are available upon request. PCR products were purified over QIAquick silica gel spin columns (Qiagen). The DNA sequence of the unique tag was determined using purified PCR product as template and an appropriate primer followed by PCR sequencing using the Perkin Elmer Dye Terminator Cycle Sequencing system with AmpliTaq DNA Polymerase and run on an Applied Biosystems 3730 DNA Analyzer (Duke DNA Sequencing Facility).

Suppression of BRCA1-induced G1 arrest and lethality. Diploid WT, *spt4Δ* and *spt5* C terminal truncation strains containing the high copy *GAL*::BRCA1 expression plasmid were grown to stationary phase (2 days at 30°C) in 200 ul of GLU-URA in 96 well plates to repress BRCA1 expression and maintain plasmid selection. Serial 5 fold dilutions were made and aliquots were replica plated to GLU-URA (BRCA1 repression) and GAL-URA (BRCA1 expression) solid media as described above. Positions within the cell cycle can be morphologically distinguished in yeast (unbudded cells are in G1; the beginning of S phase is marked by bud emergence). To examine suppression of the BRCA1-induced checkpoint delay at G1, WT and *spt4Δ* strains containing the BRCA1 plasmid were grown to logarithmic phase (1-2 x 10^7 cells/ml) in liquid GLU-URA medium. Single unbudded (G1) cells were micromanipulated into a 4 x 5 grid pattern of cells within one field of view (at 400x magnification) onto solid GLU-URA and GAL-URA plates using a Singer MSM 300 dissecting

microscope. Cell cycle progression was determined by repeatedly photomicrographing plated cells at hourly intervals.

Exposure to DNA damaging agents. WT, *spt4Δ* and terminally truncated *spt5* strains were grown to stationary phase at 30° (2 days) in 200 ul of YPD liquid medium in 96 well dishes. The cells were serially diluted in YPD (5 fold) and replica plated to YPD plates alone or YPD plates containing either 0.02% methyl methanesulfonate (MMS) or 200mM hydroxyurea (HU) using the 48 pin replica plating device described above. Cells plated to YPD alone were left unexposed. Cells were continually exposed to HU or MMS, by incubating the plates for 2 days at 30°. To generate γ-ray survival curves, WT, *spt4Δ* and *spt5* truncation strains were grown with vigorous shaking to stationary phase (4 days at 30°C) in liquid YPD. Cells were washed and resuspended in sterile water and exposed to 40, 80 or 120 krads of IR at a dose rate of 2.38 krads/min. Cell dilutions were subsequently plated to YPD and incubated at 30°C for 3-4 days. The number of viable colonies at a given dilution was used to determine relative survival fractions of colony forming ability.

***DIN*::LacZ DNA damage signaling assay.** WT, *ccr4Δ, spt4Δ* and *dhh1Δ* diploid strains were transformed with the plasmid pJS23 (*DIN*::LacZ). This selectable (*LEU2*) low copy plasmid has the *d*amage *in*ducible promoter (DIN) from *RNR3* (*r*ibo*n*ucleotide *r*eductase) fused to the β-galactosidase gene (LacZ). WT, *ccr4Δ, spt4Δ* and *dhh1Δ* strains containing pJS23 were patched onto SC glucose containing medium lacking leucine (GLU-LEU) medium and grown for 2 days

at 30°C. Patches of cells were transferred by replica plating to GLU-LEU +Xgal with (2 mM) or without the alkylating agent MMS in the presence of BU salts buffer [28]. Plates were allowed to grow for 2 days at 30°C and photographed. Alternatively, WT, *ccr4Δ, spt4Δ* and *dhh1Δ* strains containing pJS23 were patched directly onto GLU-LEU medium containing Xgal (with and without MMS) and grown for 2 days at 30°C. MMS damage-induced activation of *DIN*::LacZ is detected as a blue color in the presence of Xgal.

Co-immunoprecipitation of BRCA1 with anti-Spt4 antibody. WT diploid cells containing the *GAL*::BRCA1 plasmid were grown to logarithmic phase in GLU-URA liquid medium (100 ml). Cell cultures were split equally into two separate 50 ml conical centrifuge tubes, pelleted and washed 2x in sterile water. The resulting cell pellets were resuspended into either 100 ml of fresh GLU-URA or 100 ml of GAL-URA liquid media. Cells were allowed to grow with vigorous shaking for 18 hours at 30°C. Whole cell lysates were made by pelleting the cells (2 x 50 ml) and discarding the residual growth medium. Each cell pellet was resuspended in 0. 5 ml of ice cold HSB3 [36] buffer + 0.1% NP40 and the protease inhibitors (Roche; 1 tablet was dissolved in 5 ml of ice cold HSB3 buffer). Acid washed glass beads (0.1 ml) were added to the resuspended pellets in a microfuge tube and the cells were lysed by sequentially vortexing each sample (4 x) for 1 minute pulses. Lysates were kept on ice between pulses. Whole cell lysates were centrifuged and the supernatant fluid was equally divided such that half the volume (0.7ml) was mixed in a microfuge tube with 5 ul (1 ug) of anti-yeast Spt4 antibody (sc-26353; Santa Cruz) plus 30 ul of agarose G beads. To the other half was added 10 ul (1 ug) of anti –BRCA1 antibody (AB-1; Calbiochem) plus 30 ul of an equal 1:1 mixture of agarose A + G beads. IP

reactions were mixed by slow rotation overnight in the cold (4°C). IP reactions were spun down at 3500 rpm and the supernatant fluid was carefully aspirated and discarded. IP reactions were washed 3x in ice cold HSB3 buffer (500 ul) without protease inhibitors. Following the last wash, 30 ul of 2x loading buffer was added to the tube and heated at 95°C for 10 minutes. The bottom of the microfuge tube was pierced with a tuberculin syringe and the loading buffer is spun (3500 rpm for 10 seconds) into a fresh tube leaving the agarose beads behind. The sample was then separated on a preformed gradient TRIS-HCL polyacrylamide gel (Biorad).

***In situ* yeast immunofluorescence for heterologous expression of BRCA1.** *In situ* visualization of BRCA1 expression in yeast by immunofluorescence (IF) was adapted from the previously described methods [37]. The diploid WT and *spt4*Δ yeast strains containing the *GAL*::BRCA1 expression plasmid were grown to logarithmic phase in 10 ml of GLU-URA media at 30°C. Cells were pelleted, washed and resuspended in 10 ml sterile water. Five ml of the resuspended cells were added to 4 ml of 2X-sugarless SC medium lacking uracil and either 1 ml of 20% galactose or 1 ml of 20% glucose. The cells were induced for BRCA1 expression (in GAL-URA) by shaking for six hours at 30°C. One ml of a 37% formaldehyde solution was added to the cell suspensions and fixed at room temperature for one hour with gentle agitation. The fixed cells were recovered by centrifugation and resuspended in 2.0 ml of 50 mM phosphate-buffered formaldehyde plus sorbitol (1.2 M) and fixed a further two hours at room temperature. The cells were recovered by centrifugation and washed twice in solution B (1 M K_2HPO_4 with 1 M KH_2PO_4; pH 7.5). The solution was diluted with water to 100 mM and sorbitol was added to a final concentration of 1.2 M. The pellets were

resuspended in 1 ml solution B containing 2 μl of 2-mercamptoethanol and 20 μl of Zymolyase stock (1mg/ml Zymolyase 100T in water) and incubated at 37°C for 30 minutes with gentle agitation. The cells were recovered by centrifugation, washed and resuspended in 2.0 ml of solution B. The cells were stored at 4°C until antibody staining.

Polylysine (40 ul of 1 mg/ml polylysine solution; Sigma # P-1524) was added to the center of individual wells within an 8 well tissue culture slide. After 10 seconds, the wells were aspirated to dryness and washed three times with sterile water. After the final wash, wells were aspirated to dryness and rewashed with sterile water for 10 minutes. Fifteen μl of the resuspended cells were placed in the center of each well. After one minute, the cells were again aspirated to dryness, washed once in PBS, aspirated and allowed to air dry for 10 minutes. The cells were fixed at -20°C in methanol and acetone for 5 minutes and 30 seconds, respectively. The cells were exposed to blocking buffer (5% normal goat serum, 1% glycerol, 0.1% BSA, 0.1% fish skin gelatin in sterile PBS) for 30 minutes and aspirated to dryness and 50 μl of the primary antibody, (Ab-1 anti-BRCA1antibody diluted 1:100 in blocking buffer) was added to each well and allowed to sit at room temperature for 2 hours. Normal mouse primary antibody was added as a negative control. The cells were aspirated to dryness and washed three times in PBS. The secondary antibody (1:500 Alexafluor 488 goat anti-mouse antibody was diluted in blocking buffer) and allowed to conjugate on the slide at room temperature for 1 hour. The cells were aspirated to dryness and washed three times with PBS as described above. Hoechst 33342 stain diluted 1:10,000 in PBS was added and allowed to stain cellular DNA for 5 minutes. The cells

were aspirated to dryness and washed once with sterile water for two minutes. Vecta stain was added to the slides and a cover slip was mounted over the IF stained cells.

Results

Identification of a genetic interaction between BRCA1 and Spt4 using a whole-genome yeast screen. BRCA1 has been found to physically interact with many proteins of diverse function in human cell. Although a BRCA1 ortholog is not found in the yeast *S. cerevisiae*, over expression of BRCA1 in yeast (or human cells) results in lethality. This suggests that BRCA1-induced lethality in yeast may result from BRCA1 interacting inappropriately with highly conserved orthologs of the BRCA1 tumor suppressor pathway in yeast. In order to find a conserved functional role for the pleiotropic tumor suppressor BRCA1, we screened the isogenic diploid yeast deletion collection for null deletions that would suppress the lethality induced by heterologous expression of BRCA1 from a high copy selectable plasmid. In this screen full-length BRCA1 was fused to the yeast *GAL1* promoter such that expression of BRCA1 is repressed in cells grown in glucose containing medium and the plasmid containing the *GAL*::BRCA1 fusion is maintained intact. Following growth of WT cells containing the BRCA1 plasmid in galactose containing medium, BRCA1 is induced which results in a prolonged G1 arrest and lethality. We reasoned that an interaction of BRCA1 with a conserved yeast protein(s) interfered with a basic cellular function essential for cellular survival. Therefore, absence of the interacting protein due to deletion (*i.e* among strains in the yeast deletion collection) would result in enhanced survival following BRCA1 expression. By screening for rapid growth on GAL-URA medium immediately following transformation of the *GAL*::BRCA1 fusion plasmid into a

pool of genetically tagged deletion strains (to enable identification of the deletion), we determined that deletion of the open reading frame *YGR064W* suppressed BRCA1-induced growth arrest. Since *YGR064W* overlaps and deletes the N terminal end of the transcription factor *SPT4* we retransformed *spt4Δ* and *ygr064wΔ* isolates obtained from the frozen diploid deletion library strain collection with the *GAL*::BRCA1 fusion plasmid and tested these strains for suppression of BRCA1 induced lethality (Fig. 1). Following establishment of the BRCA1 plasmid in these strains, we determined that both the diploid *ygr064wΔ* and *spt4Δ* strains suppressed BRCA1-induced lethality when plated to galactose to induce BRCA1 expression (Fig. 1). Spt4 interacts with Spt5 in yeast and human cells to mediate transcription elongation. Although *SPT5* is an essential gene, two overlapping ORFs in the diploid deletion collection truncate the C terminal end of *SPT5*. These deletion strains are viable and exhibit ionizing radiation sensitivity[34] suggesting a role for *SPT5* in radiation toleration in yeast. Furthermore, Spt4p has been found to physically interact with Spt5p. We, therefore, transformed these two *SPT5* truncation mutants (*spt5-T*) with the *GAL*::BRCA1 plasmid and also found suppression of BRCA1-induced lethality when compared to WT. Although, the two *SPT5* truncation deletion strains were not as effective in rescuing BRCA1-induced lethality when compared to strains containing deletions of *SPT4* (Fig. 1) these results suggest that suppression of BRCA1-induced lethality was due to the absence of *SPT4* and not the undefined ORF *YGR064W*. Similar results for the involvement of *SPT4* in transcription coupled repair could be attributed to loss of *SPT4* and not to the loss of *YGR064W* [38]. These results also suggest that BRCA1 may physically interact with Spt4p to induce growth arrest in yeast.

Spt4 is required for G1 arrest and lethality following BRCA1 expression. We have previously described a prolonged G1 arrest and lethality in WT diploid yeast following BRCA1 expression. This G1 arrest and lethality could be suppressed by deletion of either *DHH1* or *CCR4*, two interacting components of the CNOT damage response network [28 39]. In budding yeast, positions within the cell cycle are morphologically determined. Single unbudded cells are in the G1 phase of the cell cycle, cells with a small bud are in S phase while cells containing a bud half the size of the mother cell (or larger) are in the G2/M phase. We examined single unbudded (G1) *spt4Δ* cells from a logarithmically growing culture for cell cycle progression following BRCA1 induction. Deletion of *SPT4* suppressed the G1 arrest following plating to GAL-URA (BRCA1 expression) when compared to WT on the same plate (Fig. 2). Following 24 hours of growth on GAL-URA plates, all (20/20) of the WT cells containing the *GAL::*BRCA1 plasmid remain arrested as single unbudded cells (*i.e.* in G1). In the *spt4Δ* strain containing the *GAL::*BRCA1 plasmid, most (13/20) of the G1 cells had progressed into microcolonies of 2 or more cells. Interestingly, WT cells containing the BRCA1 exhibit a delay in growth when plated to GLU-URA when compared to the *spt4Δ* strain growing on the same plate. These results suggest that under growth conditions that represses *GAL::*BRCA1 expression, cell cycle progression of WT cells in G1 is partially delayed when compared to the *spt4Δ* strain. Thus, very low levels of BRCA1 expression (*i.e.* protein undetectable by Western Blot analysis or *in situ* immunofluorescence) may have a growth inhibitory effect on WT yeast.

Spt4 is required for resistance to DNA damage. In human cells, BRCA1 has been implicated in the repair of a variety of DNA lesions including radiation-induced DSBs. If the genetic

interaction of BRCA1 with *SPT4* and *SPT5* in yeast is representative of a conserved role for these proteins in breast cancer, we would expect that defects in these genes may confer sensitivity to DNA damaging agents that induce DSBs. We, therefore, exposed *spt4Δ* and *spt5* truncated diploid strains to increasing doses of IR and compared the dose dependent decrease in survival to that seen in the isogenic WT strain. When compared to the WT diploid strain, both the *spt4Δ* and the *spt5-T* strains demonstrated significantly enhanced sensitivity to the lethal effects of IR at all of the doses examined (Fig. 3A). These strains both demonstrated a "non-shouldered" dose-dependent decrease in survival as compared to the WT strain which produced a radioresistant "shoulder" at 40 krads followed by a dose dependent decrease in survival at 80 and 120 krads (Fig. 3A). Furthermore, the sensitivity of the *spt4Δ* and *spt5-T* strains to IR were virtually identical suggesting that both proteins may be required for the same molecular mechanism of IR resistance. Although the relative IR sensitivity of the *spt4Δ* and *spt5-T* strains (which are defective in transcriptional elongation) was not as great as that for the *ccr4Δ* strain (Fig 3A), the IR sensitivity was very similar to that expressed among isogenic strains deleted for other transcriptional regulators (*i.e. srb5Δ*) contained within the Ccr4 damage response network [39].

Since deletion of other members of the Ccr4 damage response network (*i.e. ccr4Δ* and *dhh1Δ*) render cells sensitive to chemical agents that induce DSB damage in S phase [34], we similarly exposed the *spt4Δ* and *spt5-T* strains to the S phase specific DNA damaging agents hydroxyurea (HU) and methyl methanesulfonate (MMS). When exposed to HU, the *spt4Δ* and the *spt5* truncated strain *yml009w-bΔ* showed enhanced sensitivity to the growth inhibitory effects of this

agent when compared to WT (Fig. 3B). Interestingly, the shorter truncation of *SPT5* (*yml009c-aΔ*) did not show sensitivity to HU or MMS even though it was detected as being IR sensitive in our genetic screen [34]. None of the *spt4Δ* or *spt5-T* strains appeared to be MMS sensitive at the lowest dose tested (2 mM) which was sufficient to induce moderate lethality in the *ccr4Δ* strain (data not shown). However, the *spt4Δ* strain did show enhanced sensitivity to MMS when compared to WT when the dose was increased to 0.02% (Fig. 3B) or 0.03% (data not shown). Although the lethal effects seen in the diploid *spt4Δ* strain following exposure to HU or MMS was not as great as that seen for the isogenic diploid *ccr4Δ* strain (Fig. 3B), these results suggest that similar to *ccr4Δ*, the *spt4Δ* strain is sensitive to replication stress induced during S phase. Thus, the conserved RNA pol II cofactor Spt4 and Spt5 that physically interact together in a complex to facilitate transcription elongation are also required to mediate resistance to IR and HU both of which are capable of inducing DNA DSBs. Moreover, this suggests that the genetic screen we used to detect the *spt4Δ* as a suppressor of BRCA1-induced lethality is biologically relevant since we have identified a protein involved in the maintenance of genomic integrity (*i.e.* resistance to IR), which is also a BRCA1-dependent process.

***SPT4* deletion strains are defective in *d*amage-*in*ducible expression of *DIN*::LacZ.** Deletion of *CCR4* was previously found to suppress the G1 arrest and lethality following BRCA1 expression in yeast. Similar to the *spt4Δ* diploid strain, *ccr4Δ* mutants are sensitive to IR and HU but fail to induce the expression of the *d*amage *in*ducible *DIN*::LacZ reporter plasmid in the presence of MMS (Fig 4A). We similarly exposed the *spt4Δ* strain containing the *DIN*::LacZ plasmid to the alkylating agent MMS and determined that like *ccr4Δ* strains, the *spt4Δ* strain

failed to induce expression of the *DIN*::LacZ reporter plasmid (*i.e.* turn blue) in the presence of Xgal (a chromophoric substrate of β-galactosidase) and MMS (Fig. 4A). The *DIN*::LacZ plasmid contains the promoter from ribonuclease reductase (*RNR3*) fused to the β-galactosidase reporter gene. Since *RNR3* is under the control of the *DUN1* kinase, which is a terminal effector of the *RAD9*-dependent DNA damage checkpoint pathway, this suggests that similar to Ccr4, Spt4 may be required for DNA damage signaling. Alternatively, transcriptional elongation may be impaired in the presence of MMS-induced DNA damage within the LacZ coding region in the *spt4Δ* and *ccr4Δ* mutants. Since no activation of the *DIN*::LacZ plasmid was observed spontaneously (in the absence of DNA damage) for the *spt4Δ* strain (Fig. 4A), this suggests that *spt4Δ* may be defective in transcriptional elongation of G-rich sequences such as those contained within the β-galactosidase gene (see Discussion). Since BRCA1 is also a relatively G-rich human gene, we determined whether, *spt4Δ* strains were similar to WT in their ability to synthesize BRCA1 protein, which would indicate that transcription and subsequent mRNA transport to the cytoplasm for translation is unimpaired. We, therefore, induced BRCA1 expression in WT and *spt4Δ* strains and used indirect *in situ* IF to determine the relative ability of these strains to synthesize BRCA1 protein product. Using IF, both WT and *spt4Δ* strains were found to produce BRCA1 protein in similar amounts (Fig. 4B) suggesting that defects in transcriptional elongation in the *spt4Δ* strain does not prevent synthesis of BRCA1 protein in the absence of DNA damage. Thus, the rescue of BRCA1-induced lethality in the *spt4Δ* strain does not result from down regulation of BRCA1 transcription resulting in a concomitant decrease in production of the BRCA1 protein product.

Spt4p physically interacts with BRCA1. Suppression of BRCA1-induced lethality in *spt4Δ* strains suggests that Spt4p may be an interactive target to which the BRCA1 protein physically binds. We, therefore, prepared whole cell lysates from WT cells following BRCA1 expression and subjected the crude protein extract to co-IP using anti-Spt4p antibody. Whole cell lysates and co-IP reactions were separated on gradient polyacrylamide gels and BRCA1 protein was detected by Western blotting (Fig. 5). Similar to previously published results [28], full length BRCA1 product (>220 kDa) could be detected in crude cell extracts following 16 hr of BRCA1 expression in GAL-URA liquid medium. However, as previously described the majority of BRCA1 protein appeared as lower molecular weight products suggesting that proteolytic degradation may have occurred. BRCA1 protein product (full-length or degradation products) could not be detected in crude lysates from cells in which BRCA1 was repressed (*i.e.* those grown in GLU-URA). Using anti-Spt4p antibody, full length BRCA1 was detected in two independent co-IP reactions from separate galactose-induced WT isolates containing the *GAL*::BRCA1 plasmid (Fig. 5). The intensity of the co-IP BRCA1 bands were similar to that obtained by immunoprecipitation of the identical whole cell lysates using anti-BRCA1 antibody. Furthermore, the intensity of the full-length co-IP bands were greater than that observed from the crude lysate. This suggests that the anti-Spt4 antibody has reacted with all of the available full-length BRCA1 product. Although some BRCA1 degradation products can be seen in the co-IP reactions, BRCA1 interaction with Spt4p appears to occur preferentially with the full-length product. As expected, co-IP reactions using anti-Spt4 or anti-BRCA1 antibodies and whole cell lysates from glucose grown cells failed to produce any detectable BRCA1 product (Fig. 5). Furthermore, co-IP reactions using none-immune IgG from goat or mouse also failed to co-IP any detectable high molecular weight full length BRCA1 product from galactose grown cell

extracts (data not shown). These results indicate that endogenous levels of Spt4p are sufficient interact with BRCA1 when expressed in WT diploid yeast and mediate co-IP of BRCA1 using anti-Spt4 antibody.

Discussion

The yeast *Saccharomyces cerevisiae* has served as an important model organism for the elucidation of complex, highly conserved genetic pathways including those that maintain genetic stability following DNA damage. In fact, most of the highly conserved human repair proteins involved in DSB repair were first identified in yeast [26]. The tumor suppressor BRCA1 plays diverse and complex roles in maintaining genome stability following DNA damage, especially DNA DSBs. Furthermore, inheriting a defective BRCA1 allele results in an enhanced lifetime risk of contracting breast cancer [40]. Initiation of breast cancer disease is thought to result from the inability to maintain genomic integrity following the loss of heterozygosity at the BRCA1 locus. Thus, the identification of new interactive BRCA1 targets that contribute to the maintenance of genetic stability is of paramount importance for the elucidation of the molecular mechanism of breast cancer. We, therefore, used a functional genomic approach in the genetically accessible simple eukaryote *S. cerevisiae* to identify potential new interactive targets of the human tumor suppressor BRCA1. Although yeast lack an ortholog of BRCA1, overexpression of this protein leads to prolonged G1 arrest and lethality in WT diploid yeast [28]. We reasoned that this lethality resulted from BRCA1 interacting inappropriately with evolutionarily conserved components of DNA repair pathways required to maintain genomic stability following DNA damage. Therefore, deletions that suppressed or rescued BRCA1-induced lethality would be predicted to be both highly conserved and confer resistance to DNA

damaging agents that induce DSBs, such as IR. These criteria appear to be satisfied in the case of *SPT4* which confers resistance to IR, HU and MMS and is highly conserved in all higher eukaryotes including humans.

Mutants of *SPT4* (*sup*pressor of *T*y) were first isolated as a suppressor of insertion mutations where the transposable element Ty was inserted into the 5' region of the *HIS4* gene that down regulated expression [41]. These authors found that one haploid mutant allele of *SPT4* (*spt4-3*) was sensitive to MMS as did another study [38] thus confirming our finding of sensitivity of the diploid *spt4Δ* strain to high doses of MMS. Spt4p has been found to interact with Spt5p in a complex to mediate transcription elongation functions in human [25] and yeast cells [42]. Furthermore, a role for SPT4 in transcription coupled repair (TCR) has been suggested since the absence of *SPT4* suppressed the TCR defect found in a *rad16rad26* mutant following UV treatment [38]. Given our results with *spt4Δ* strains containing the *DIN*::LacZ plasmid in the presence or absence of MMS, Spt4 may be required to bypass spontaneous or MMS-induced DNA lesions during transcription elongation. Such transcription elongation difficulties within the G-rich LacZ sequence have been previously documented [43]. Furthermore, enhanced transcription mediated recombination or plasmid loss (in a plasmid containing a *GAL*::LacZ construct) in a *spt4Δ* strain compared to WT suggests that transcription may induce DNA DSB damage in *spt4* strains. Therefore, in the absence of Spt4p (or Ccr4p), MMS-induced DNA lesions could completely block RNA pol II elongation within the β-galactosidase coding region resulting in the observed white phenotype in the presence of the Xgal substrate. RNR3p and many other gene products that are required to prepare for cell cycle dependent entry into S phase are transcribed prior to DNA synthesis in late G1. Thus, a transcript containing a RNA pol II complex blocked at a lesion may result in an

impediment to DNA polymerase progression. If this is left unresolved (*i.e.* unrepaired) it could result in lethality due to replication-induced DNA breakage as cells progress through S phase.

In a similar manner, heterologous BRCA1 expression appears to result in loss of the high copy *GAL*::BRCA1 plasmid as indicated by enhanced loss of the *URA3* plasmid marker or the plasmid itself following galactose-induced expression of BRCA1 in WT cells. This loss is completely suppressed in *spt4Δ* cells (data not shown). Since BRCA1 physically binds to Spt4p in yeast cells, it is tempting to speculate that BRCA1 may bind to Spt4p inappropriately to arrest transcription, which in turn elicits G1 arrest and lethality due to plasmid and/or chromosomal loss. Conversely in human cells, Spt4 may be required to arrest transcription in the presence of DNA damage to initiate TCR. The finding that expression of BRCA1 in BRCA1-defective cancer cells can restore resistance to IR by enhancing TCR supports the possible involvement of Spt4 in mediating this process [44]. Thus, BRCA1 may interact with Spt4 in human cells to signal the presence of and/or initiate the repair of DNA damage by TCR. Rescue of the BRCA1-induced lethality does not appear to result simply from transcriptional down regulation of BRCA1 expression since we observed levels of protein expression similar to WT when cells were prepared for IF detection of BRCA1 protein. Furthermore, many IR sensitive mutants in the same transcription pathway as *CCR4* failed to significantly rescue BRCA1-induced lethality such as *not3, not4 (mot2), not5* or *pop2* (data not shown). Therefore, specific interaction with Spt4p, Ccr4p or Dhh1p appears to be required to observe BRCA1-induced lethality.

BRCA1 has been shown to specifically interact with the RNA polymerase II holoenzyme complex [6]. Furthermore, the BRCT domain of BRCA1 was shown to interact with the

holoenzyme complex by specifically interacting with RNA helicase A [17]. Moreover, BRCA1 appears to associate preferentially with the processive form of RNA pol II that was hyperphosphorylated in the catalytic subunit of undamaged cells [18]. Upon treatment with DNA damaging agents, this association was disrupted suggesting a link between DNA damage signaling/repair and transcription [18]. BRCA1 has also been shown to interact with the NUF1P and P-TEFb two co-regulators of transcription [45]. The positive elongation factor P-TEFb is thought to mediate the transition from RNA initiation to elongation by phosphorylation of the CTD (C terminal domain) region of RNA pol II [46]. NUF1P appears to interact with the template throughout the transcription cycle and facilitates the removal of hyperphosphorylated forms of RNA pol II from open transcription complexes [45]. BRCA1 has also been found to interact with the negative elongation factor NELF-B/COBRA1 which is part of the NELF complex that interacts with the DSIF (5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole sensitivity inducing factor) complex to negatively regulate RNA pol II transcription before the action of P-TEFb promotes elongation [23]. Perhaps not surprisingly, the DSIF complex has been found to be composed of human Spt4-Spt5 [24] which are the orthologs of the yeast Spt4p and Spt5p described in this study. Thus, BRCA1 appears to interact with both positive and negative regulators of transcription which may account for its ability to either activate or repress transcription depending on the target and experimental conditions employed [47] [40].

Emerging evidence suggests that eukaryotic cells may employ the RNA pol II holoenzyme complex and transcription for DNA damage surveillance [48] [49] [19]. In this respect, BRCA1 may act as a sensor of DNA damage that monitors stalling of the transcription apparatus at DNA lesions

and, therefore, participates in a transcriptional checkpoint to signal the presence of lesions within active transcriptional regions [50]. In the response to DNA damage, BRCA1 has been implicated both upstream as a damage sensor or signaling protein and downstream as a transcriptional regulator of DNA damage response genes [40]. Both of these roles could be accommodated in a model that has BRCA1 interacting with both negative regulatory cofactors of RNA pol II (such as Spt4 or NELF-B) to mediate damage sensing whereas interaction of BRCA1 with positive transcription cofactors could promote the transcription of damage response genes independent of p53 [51]. The identification of the evolutionarily conserved Spt4 as an interactive partner of BRCA1 in yeast suggests BRCA1 may similarly utilize the transcription elongation apparatus to sense DNA damage in human cells. Therefore, this process may be essential for maintaining genome integrity and suppressing the onset of breast cancer.

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Legends

Figure 1. Deletion of *SPT4* suppresses BRCA1-induced lethality in yeast. The diploid deletion strain *ygr064WΔ* was initially identified in a screen to identify rapidly growing strains on synthetic complete (SC) medium containing galactose (GAL) but lacking uracil (URA) following transformation of a high-copy *GAL*::BRCA1 into a pool of 4653 "tagged" diploid deletion strains. The plasmid contains *URA3* as a selectable marker. The *ygr064WΔ* strain was obtained from the diploid deletion collection arrayed in 96 well dishes and retransformed with the *GAL*::BRCA1 plasmid. Pictured above is the relative survival of this retransformed deletion strain when plated to SC glucose (GLU) – URA and SC GAL-URA. Since the *ygr064WΔ* deletes the N terminus and promoter region of *SPT4,* we obtained the complete deletion of *SPT4* (*spt4Δ)* from the deletion collection and established the *GAL*::BRCA1 plasmid within this strain by transformation. Deletion of *SPT4* completely suppressed BRCA1-induced lethality. Spt4p physically interacts with Spt5p to confer transcription elongation functions. Since *SPT5* is an essential gene, we examined the relative survival of two strains (*yml009W-bΔ* and *yml009c-aΔ*) containing non-lethal C terminal truncations of *SPT5* on GLU-URA *vs* GAL-URA following establishment of the of the *GAL.:*BRCA1 plasmid by transformation. Defects in *SPT5* also suppress BRCA1-induced lethality suggesting transcription elongation is inhibited by BRCA1 in yeast. The relative plating efficiencies of these strains (GAL/GLU) have been indicated (each data point is the average of 3 or more individual experiments). The fold-increase in relative plating efficiencies for the mutant strains were 765x (*ygr064wΔ*), 810x (*spt4Δ*), 175x (*yml009w-bΔ*) and 220x (*yml009c-aΔ*). These fold increases were obtained by normalizing to the relative survival seen in the wild type strain (WT = 1.0x).

Figure 2. Deletion of *SPT4* suppresses prolonged G1 arrest following BRCA1 induction in yeast. WT and *spt4Δ* diploid cells containing the BRCA1 plasmid were grown to logarithmic phase (~2 x 10^7 cells/ml) in synthetic complete (SC) GLU-URA to repress BRCA1 expression. Individual unbudded (G1) cells of uniform size were picked using a micromanipulator and placed into 4 x 5 cell grid patterns on GLU-URA and GAL-URA plates. Cells were photographed at hourly intervals to monitor cell cycle progression. Typical results for one of three independent experiments are depicted following 24 hours of growth at 30°C. None of the WT cells expressing BRCA1 (on GAL-URA medium) were able to progress beyond the single cell (G1) stage of the cell cycle. However, deletion of *SPT4* allowed most of the cells (13/20) to progress into microcolonies following BRCA1 expression on GAL-URA plates. When plated to GLU-URA (BRCA1 repressed), most of the WT and the *spt4Δ* cells progressed beyond the G1 stage of the cell cycle to form microcolonies. Interestingly, the growth rate of the *spt4Δ* strain containing the *GAL::*BRCA1 plasmid was enhanced (*i.e.* produced larger microcolonies) as compared to the WT on GLU-URA plates suggesting that the *GAL::*BRCA1 fusion may "leak" in the presence of glucose. Therefore, low levels of BRCA1 that is undetectable by immunofluorescence or Western analysis (see Figs. 4 and 5) may be expressed in glucose grown WT cells resulting in growth inhibition on GLU-URA plates.

Figure 3. Deletion of *SPT4* results in sensitivity to agents that induce DNA double-strand break damage. Panel A. Diploid *spt4* and *spt5* strains are sensitive to γ-irradiation. WT, *spt4Δ* and *spt5* truncated (*yml009w-bΔ)* diploid strains were grown to stationary phase, washed in water and exposed to γ-irradiation as previously described. The γ-ray dose-dependent decrease in survival for the WT strain was identical to that previously described [39]; therefore, new WT

survival data was pooled with previous data (n = 10 experiments) to generate the average WT survivals depicted above. The survival of the *spt4Δ* and *spt5* truncated strains are the average of 3-4 replica experiments. The γ-ray survival of the *ccr4Δ* strain is included for comparative purposes and has been previously published [39]. Error bars are ± 1 standard error about the mean. Panel B. *spt4Δ* strains are sensitive to the S phase specific DNA damaging agents HU and MMS. WT, *spt4Δ*, *spt5* truncated (*yml009w-bΔ*; *yml009c-aΔ*) and *ccr4Δ* strains were grown to stationary phase in YPD liquid and 2ul aliquots of serial 5 fold dilutions in YPD were spotted onto control (YPD) or hydroxyurea (HU) and methyl methanesulfonate (MMS) YPD plates at the indicated concentrations. Downward slope of the triangles indicate the direction of decreasing cell concentrations. The *ccr4Δ* strain has been included as a positive control since it was previously found to be HU and MMS sensitive [39].

Figure 4. *SPT4* deletion strains are defective for damage-induced expression of a *DIN*::LacZ reporter plasmid. Panel A. Diploid WT, *ccr4Δ*, *dhh1Δ* and *spt4Δ* strains were transformed with a selectable (*LEU2*) *DIN*::LacZ plasmid where the promoter of the damage inducible ribonuclease reductase gene (*RNR3*) was fused to β-galactosidase. If the *DIN*::LacZ plasmid is activated (spontaneously or by DNA damage), enhanced transcriptional activity is detected as the production of a blue color in the presence of Xgal. Following exposure to MMS (+MMS, 2 mM), the radiation (and MMS) sensitive *ccr4Δ* and *spt4Δ* strains that also suppress BRCA1-induced lethality fail to activate the expression of *DIN*::LacZ. However, the *dhh1Δ* strain that is also IR (and MMS) sensitive and suppresses BRCA1-induced lethality shows enhanced activation of *DIN*::LacZ following exposure to MMS (*i.e.* turned dark blue) indicating that defects in transcriptional activation or elongation is not a conserved feature of radiation

sensitive mutants that rescue BRCA1-induced lethality. Since the WT strain is not sensitive to MMS at the dose examined, little or no MMS-induced activation of *DIN*::LacZ was observed. Slight backgrpond activation of the *DIN*::LacZ in the absence of DNA damage (spontaneous) was observed in the WT and *dhh1Δ* strains (indicated by a light blue color). Panel B. BRCA1 is expressed in *spt4Δ* diploid cells. Decreased transcriptional activation of *DIN*::LacZ in *spt4Δ* strains suggests that transcriptional initiation or elongation of some transcripts may be inhibited. We therefore compared the level of BRCA1 protein expression in WT *vs spt4Δ* diploid cells using *in situ* immunofluorescence (IF) of permeabalized yeast cells induced for 6 hours in GAL-URA liquid medium (BRCA1 expressed). Both the WT and *spt4Δ* strains produced similar quantities of BRCA1 protein as determined by indirect IF of fixed yeast cells using anti-BRCA1 antibody (AB-1) as a probe. BRCA1 was determined to be both nuclear and cytoplasmic as the position of the yeast nuclei could be determined using the DNA specific Hoechst strain. Little or no background BRCA1 staining was observed in the cells maintained in GLU-URA medium (BRCA1 repressed). These results indicate that the suppression of BRCA1-induced lethality in *spt4Δ* cells was not due to decreased transcriptional expression of BRCA1.

Figure 5. Spt4p physically interacts with BRCA1 in yeast cells. We used co-immunoprecipitation of BRCA1 with anti-Spt4p antibody to demonstrate BRCA1 interacts physically as well as genetically (see Fig. 1) in yeast. BRCA1 was expressed in a diploid WT yeast strain containing the inducible *GAL*::BRCA1 fusion plasmid construct. Plasmid bearing cells were grown to logarithmic phase (~2 x 10^7 cells/ml) in synthetic complete (SC) glucose medium lacking uracil (GLU-URA) to repress expression of BRCA1. Cells were washed and grown for 16 hours in SC galactose (GAL-URA) to induce expression of BRCA1. Crude

extracts were either loaded directly (Western) or subjected to co-immunoprecipitation (co-IP) overnight using anti-Spt4p antibody (sc-26353, Santa Cruz) or anti-BRCA1 antibody (AB-1, Calbiochem). For both antibodies, two separate co-IP reactions using different whole cell lysates of WT diploid yeast induced in galactose are depicted. Co-IP protein complexes were run on a gradient TRIS-HCL polyacrylamide gel (Biorad) and transferred to nitrocellulose. The membrane was subjected to Western blotting using anti-BRCA1 antibody (AB-1) as a probe. Full length BRCA1 is detected as a single band in extracts of WT cells exposed to galactose but not to glucose (arrows) migrating above the 220 kDa myosin band in the Rainbow protein molecular weight marker (Amersham) ladder lanes (L). This BRCA1 band was detected in both co-IP reactions using whole cell lysates from 2 separate galactose-induced cultures. Galactose induction of BRCA1 in WT cells produces both full-length product and a "smear" of low molecular weight BRCA1 fragments as previously described [28]. No full-length BRCA1product was detected in co-IP reactions using whole cell lystes from galactose induced cells and non-immune mouse or goat IgG (data not shown). The cross-reacting IgG heavy chain (HC) bands have been indicated for reference.

Figure 1

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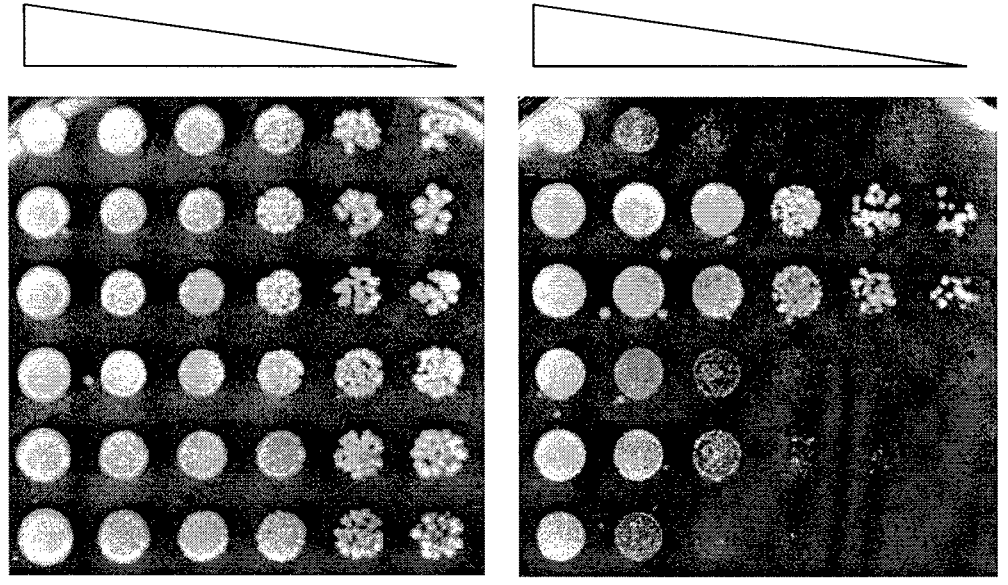

***Spt5 truncation**

Figure 2

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SPT4 deletion suppresses BRCA1-induced G1 arrest

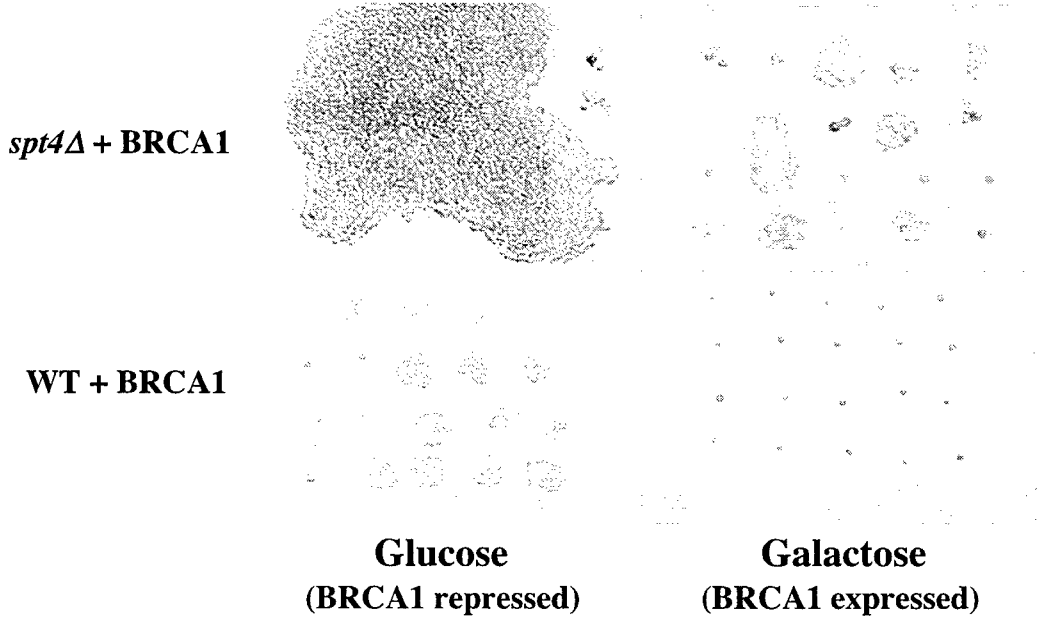

Figure 3

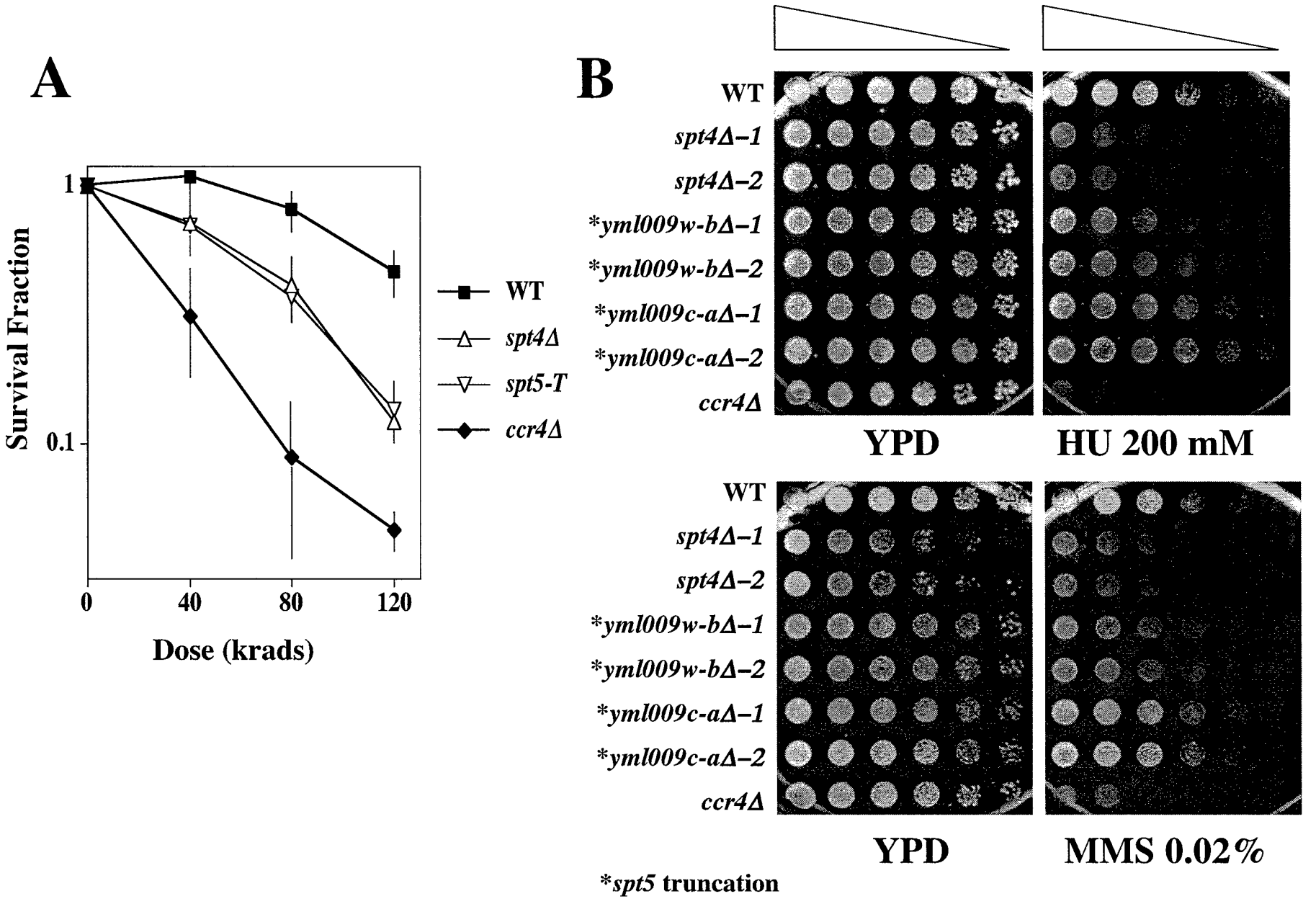
A
Survival Fraction
1
0.1
0
40
80
120
Dose (krads)
WT
spt4Δ
spt5-T
ccr4Δ
B
WT
spt4Δ–1
spt4Δ–2
*yml009w-bΔ–1
*yml009w-bΔ–2
*yml009c-aΔ–1
*yml009c-aΔ–2
ccr4Δ
YPD
HU 200 mM
WT
spt4Δ–1
spt4Δ–2
*yml009w-bΔ–1
*yml009w-bΔ–2
*yml009c-aΔ–1
*yml009c-aΔ–2
ccr4Δ
YPD
MMS 0.02%
*spt5 truncation

Figure 4

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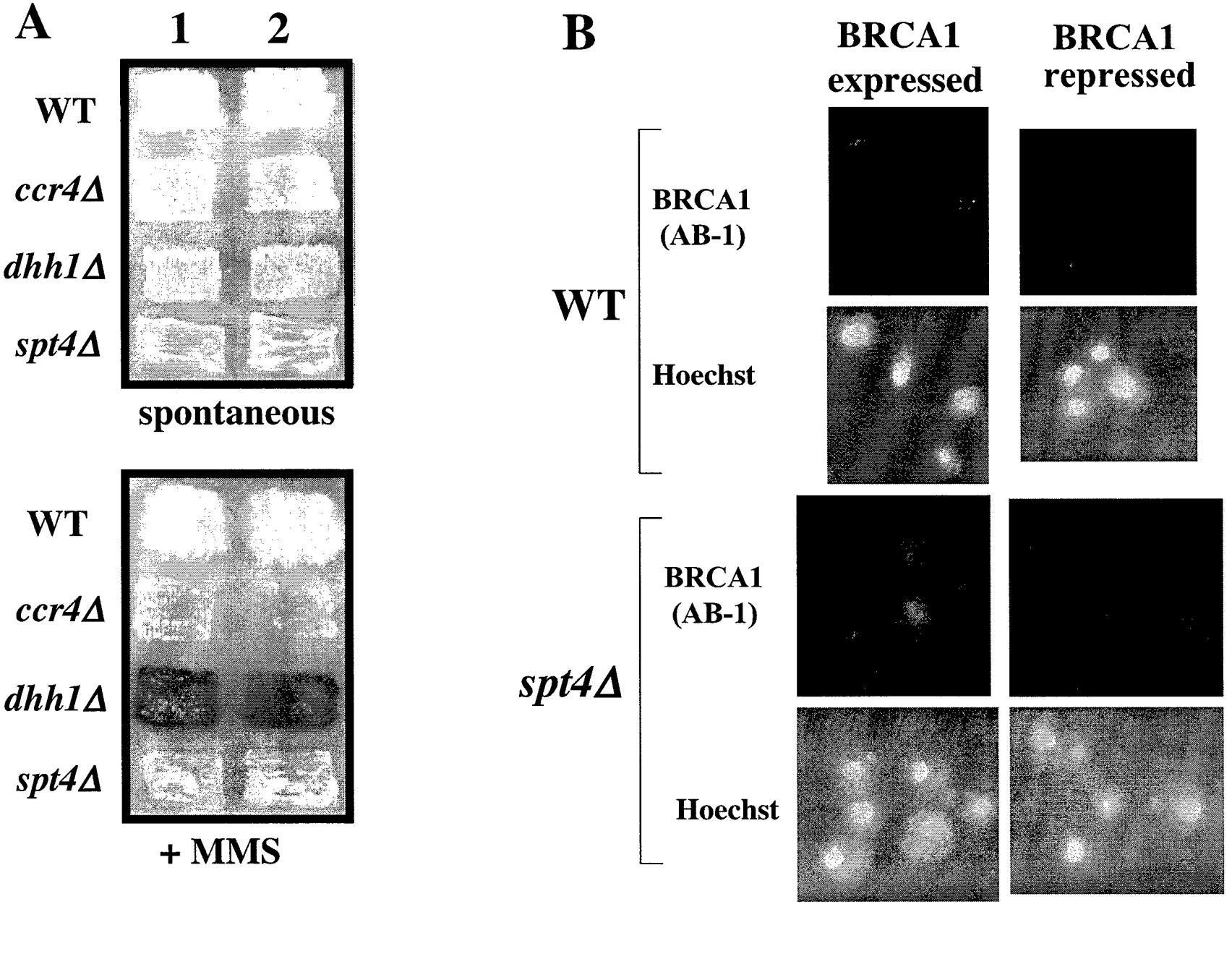

Figure 5

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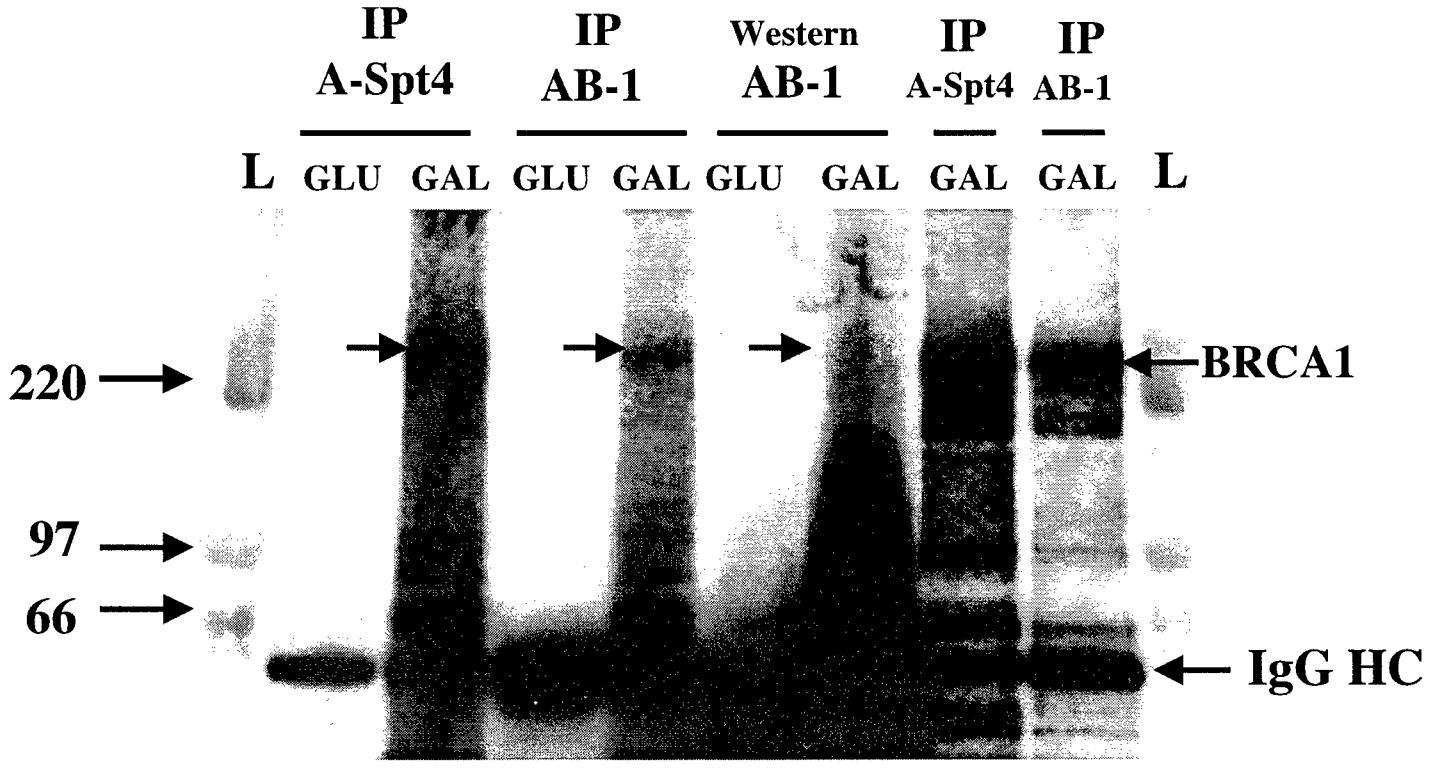

Appendix B: Zymocin sensitive diploid deletion mutants in *S. cerevisiae*

Gene	ORF	Function	Sensitivity	Dilution Plating
AAT2	YLR027C	Aspartate AminoTransferase: Cytosolic aspartate aminotransferase, involved in nitrogen metabolism; localizes to peroxisomes in oleate-grown cells; SGD after 20 & 60 generations, SGD on NaCl	3	
ABF2	YMR072W	ARS-Binding Factor: Mitochondrial DNA-binding protein involved in mitochondrial DNA replication and recombination, member of HMG1 DNA-binding protein family; activity may be regulated by protein kinase A phosphorylation; SGD after 20 & 60 generations, SGD on NaCl	1, 2	
ACF4	YJR083C	Protein of unknown function, computational analysis of large-scale protein-protein interaction data suggests a possible role in actin cytoskeleton organization; potential Cdc28p substrate; SGD after 20 & 60 generations, SGD on NaCl	2	
ACO1	YLR304C	Mitochondrial aconitase, required for the tricarboxylic acid (TCA) cycle; mutation leads to glutamate auxotrophy; MGD after 60 generations, MGD on NaCl	3, 2	SSS*
ADA2*	YDR448W	transcriptional ADAptor: Transcription coactivator, component of the ADA and SAGA transcriptional adaptor/HAT (histone acetyltransferase) complexes	2	SSS
ADE1	YAR015W	ADEnine requiring: N-succinyl-5-aminoimidazole-4-carboxamide ribotide (SAICAR) synthetase, required for 'de novo' purine nucleotide biosynthesis; red pigment accumulates in mutant cells deprived of adenine; MGD after 20 & 60 generations, MGD on NaCl	2	
ADE4	YMR300C	ADEnine requiring: Phosphoribosylpyrophosphate amidotransferase (PRPPAT; amidophosphoribosyltransferase), catalyzes first step of the 'de novo' purine nucleotide biosynthetic pathway; SGD after 60 generations	1	SS*
ADE5,7	YGL234W	Bifunctional enzyme of the 'de novo' purine nucleotide biosynthetic pathway, contains aminoimidazole ribotide synthetase and glycinamide ribotide synthetase activities	2, 2	
ADE8	YDR408C	ADEnine requiring: Phosphoribosyl-glycinamide transformylase, catalyzes a step in the 'de novo' purine nucleotide biosynthetic pathway	2, 2	S*
ADH1	YOL086C	Alcohol DeHydrogenase: involved in the production of certain carboxylate esters; SGD after 20 & 60 generations, MGD on NaCl	2, 2	
ADK1*	YDR226W	adenylate kinase; MGD after 20 & 60 generations, MGD on NaCl	2, 3	SSS
AEP1	YMR064W	ATPase ExPression: Protein required for expression of the mitochondrial OLI1 gene encoding subunit 9 of F1-F0 ATP synthase; MGD after 20 generations, SGD after 60 generations, SGD on NaCl	1, 2	
AEP2	YMR282C	ATPase ExPression: Mitochondrial protein, likely involved in translation of the mitochondrial OLI1 mRNA; exhibits genetic interaction with the OLI1 mRNA 5'-untranslated leader	1, 2	SSS, SSS*
AEP3	YPL005W	ATPase ExPression: Peripheral mitochondrial inner membrane protein, located on the matrix face of the membrane; stabilizes the bicistronic AAP1-ATP6 mRNA encoding subunits 6 and 8 of the ATP synthase complex; SGD after 20 & 60 generations, MGD on NaCl	2, 2	
AFG3	YER017C	ATPase Family Gene: Component, with Yta12p, of the mitochondrial inner membrane m-AAA protease that mediates degradation of misfolded or unassembled proteins and is also required for correct assembly of mitochondrial enzyme complexes; SGD after 20 & 60 generations, SGD on NaCl	3, 3	
AFT2	YPL202C	Activator of Iron (Fe) Transcription; SGD after 20 & 60 generations, SGD on NaCl	1	SS, SS*
AGX1	YFL030W	Alanine Glyoxylate aminotrans(X)ferase: Alanine : glyoxylate aminotransferase, catalyzes the synthesis of glycine from glyoxylate, which is one of three pathways for glycine biosynthesis in yeast; has similairty to mammalian and plant alanine : glyoxylate aminotransferases; MGD after 20 & 60 generations, MGD on NaCl	2	
AKR1*	YDR264C	AnKyrin Repeat-containing protein: Palmitoyl transferase involved in protein palmitoylation; acts as a negative regulator of pheromone response pathway; required for endocytosis of pheromone receptors; involved in cell shape control; contains ankyrin repeats; MGD after 20 & 60 generations	2	
ALF1	YNL148C	ALpha-tubulin Foldin: alpha-tubulin foldin; protein implicated in folding of alpha tubulin; cofactor B	1	SSS, SSS*
ALG5	YPL227C	Asparagine-Linked Glycosylation: UDP-glucose:dolichyl-phosphate glucosyltransferase, involved in asparagine-linked glycosylation in the endoplasmic reticulum	3	
ALG6	YOR002W	Asparagine-Linked Glycosylation: Glucosyltransferase, involved in transfer of oligosaccharides from dolichyl pyrophosphate to asparagine residues of proteins during N-linked protein glycosylation; mutations in human ortholog are associated with disease; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2	
ALG8	YOR067C	Asparagine-Linked Glycosylation: adds glucose to the dolichol-linked oligosaccharide precursor prior to transfer to protein; glycosyl transferase; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2	
ANC1* (TAF14)	YPL129W	Subunit (30 kDa) of TFIID, TFIIF, and SWI/SNF complexes, involved in RNA polymerase II transcription initiation and in chromatin modification; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	3, 3	SSS, SSS*
ANP1	YEL036C	ANP and osmotic sensitive: Mannan 8; Protein of the endoplasmic reticulum with a role in retention of glycosyltransferases in the Golgi, also involved in osmotic sensitivity and resistance to aminonitrophenyl propanediol; subunit of mannosyltransferase complex; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2	
APJ1	YNL077W	Putative chaperone of the HSP40 (DNAJ) family; overexpression interferes with propagation of the [Psi+] prion	1	SSS, SSS*
APL2	YKL135C	Beta-adaptin, large subunit of the clathrin-associated protein (AP-1) complex; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	3	
APL5	YPL195W	Delta-like subunit of the yeast AP-3 complex which functions in transport of alkaline phosphatase to the vacuole via the alternate pathway, suppressor of loss of casein kinase 1 function; delta-like subunit of the yeast AP-3 adaptin component of the membrane-associated clathrin assembly complex; MGD after 20 & 60 generations, MGD on NaCl	1	SSS, SS*
APM1	YPL259C	medium subunit of the clathrin-associated protein complex; MGD after 20 generations MGD on NaCl	2	
APN1	YKL114C	major apurinic/apyrimidinic endonuclease/3'-repair diesterase	3	
ARC18	YLR370C	ARp2/3 Complex subunit: Subunit of the ARP2/3 complex, which is required for the motility and integrity of cortical actin patches; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2	
ARF1	YDL192W	ADP-ribosylation factor, GTPase of the Ras superfamily involved in regulation of coated formation vesicles in intracellular trafficking within the Golgi; functionally interchangeable with Arf2p	3	
ARL1	YBR164C	ADP-Ribosylation factor-Like: Soluble GTPase with a role in regulation of membrane traffic; regulates potassium influx; G protein of the Ras superfamily, similar to ADP-ribosylation factor	2	
ARL3	YPL051W	ADP-Ribosylation factor-Like: Protein similar to ADP-ribosylation factor, involved in the carboxypeptidase Y pathway; MGD after 20 & 60 generations, MGD on NaCl	2	
ARO2	YGL148W	AROmatic amino acid requiring: Bifunctional chorismate synthase and flavin reductase, catalyzes the conversion of 5 enolpyruvylshikimate 3-phosphate (EPSP) to form chorismate, which is a precursor to aromatic amino acids; SGD after 20 & 60 generations, SGD on NaCl	2	
ARP1	YHR129C	Actin-Related Protein: actin-related protein of the dynactin complex; Centractin; MGD after 20 & 60 generations, MGD on NaCl	1	SSS, SSS*
ARP5*	YNL059C	Actin-Related Protein: Nuclear actin-related protein involved in chromatin remodeling, component of chromatin-remodeling enzyme complexes; SGD after 20 & 60 generations, SGD on NaCl	2	
ARV1*	YLR242C	ARE2 Required for Viability: Protein required for normal intracellular sterol distribution and for sphingolipid metabolism; similar to Nup120p and C. elegans R05H5.5 protein; MGD after 20 & 60 generations	3, 3	SSS

Appendix B: Zymocin sensitive diploid deletion mutants in *S. cerevisiae*

ATG17	YLR423C	AuTophaGy related: Protein that interacts with and is required for activation of Apg1p protein kinase; involved in autophagy but not in the Cvt (cytoplasm to vacuole targeting) pathway	2	
ATG18	YFR021W	AuTophaGy related: Phosphatidylinositol 3,5-bisphosphate-binding protein of the vacuolar membrane, predicted to fold as a seven-bladed beta-propeller; required for recycling of Atg9p through the pre-autophagosomal structure	2	SSS
ASF1*	YJL115W	Anti-Silencing Function: anti-silencing protein that causes depression of silent loci when overexpressed; involved in silencing, MGD on NaCl	3	
ATP2*	YJR121W	Beta subunit of the F1 sector of mitochondrial F1F0 ATP synthase, which is a large, evolutionarily conserved enzyme complex required for ATP synthesis	1, 2	
ATP5	YLR393W	ATP synthase: Mitochondrial inner membrane protein required for assembly of the F0 sector of mitochondrial F1F0 ATP synthase, interacts genetically with ATP6; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	3, 2	SSS*
ATP10	YDR298C	Subunit 5 of the stator stalk of mitochondrial F1F0 ATP synthase, which is a large, evolutionarily conserved enzyme complex required for ATP synthesis; homologous to bovine subunit OSCP (oligomycin sensitivity-conferring protein)	2, 3	S
ATP12	YJL180C	ATP synthase: Molecular chaperone, required for the assembly of alpha and beta subunits into the F1 sector of mitochondrial F1F0 ATP synthase	2	
ATP14	YLR295C	Subunit h of the F0 sector of mitochondrial F1F0 ATP synthase, which is a large, evolutionarily conserved enzyme complex required for ATP synthesis	2, 2	
ATP15	YPL271W	Epsilon subunit of the F1 sector of mitochondrial F1F0 ATP synthase, which is a large, evolutionarily conserved enzyme complex required for ATP synthesis	1, 2	
ATP17	YDR377W	Subunit f of the F0 sector of mitochondrial F1F0 ATP synthase, which is a large, evolutionarily conserved enzyme complex required for ATP synthesis; SGD after 20 & 60 generations, SGD on NaCl	2, 3	SS
BAS1	YKR099W	Myb-related transcription factor involved in regulating basal and induced expression of genes of the purine and histidine biosynthesis pathways	2	
BCK2	YER167W	Bypass of C Kinase: Protein rich in serine and threonine residues involved in protein kinase C signaling pathway, which controls cell integrity; overproduction suppresses pkc1 mutations; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2	
BCS1	YDR375C	Protein of the mitochondrial inner membrane that functions as an ATP-dependent chaperone, required for the assembly of the cytochrome bc(1) complex from the Rip1p and Qcr10p proteins; member of the CDC48/PAS1/SEC18 ATPase family	2, 3	
BEM1*	YBR200W	Protein containing SH3-domains, involved in establishing cell polarity and morphogenesis; functions as a scaffold protein for complexes that include Cdc24p, Ste5p, Ste20p, and Rsr1p	3	
BEM2	YER155C	Bud Emergence: Rho GTPase activating protein (RhoGAP) involved in the control of cytoskeleton organization and cellular morphogenesis; required for bud emergence; MGD after 20 generations, MGD on NaCl	1	
BEM3	YPL115C	Rho GTPase activating protein (RhoGAP) involved in control of the cytoskeleton organization; targets the essential Rho-GTPase Cdc42p, which controls establishment and maintenance of cell polarity, including bud-site assembly	2	
BEM4	YPL161C	Protein involved in establishment of cell polarity and bud emergence; interacts with the Rho1p small GTP-binding protein and with the Rho-type GTPase Cdc42p	3	
BFR1*	YOR198C	BreFeldin Resistant: Component of mRNP complexes associated with polyribosomes; implicated in secretion and nuclear segregation; multicopy suppressor of BFA (Brefeldin A) sensitivity	2	
BIK1*	YCL029C	Microtubule?associated protein, component of the interface between microtubules and kinetochore, involved in sister chromatid separation; essential in polyploid cells but not in haploid or diploid cells; ortholog of mammalian CLIP-170	3	
BIM1	YER016W	Microtubule-binding protein that together with Kar9p makes up the cortical microtubule capture site and delays the exit from mitosis when the spindle is oriented abnormally	2	
BNI1	YNL271C	Bud Neck Involved: Formin, nucleates the formation of linear actin filaments, involved in cell processes such as budding and mitotic spindle orientation which require the formation of polarized actin cables, functionally redundant with BNR1	3	
BOP2	YLR267W	Bypass Of Pam1: Protein of unknown function, overproduction suppresses a pam1 slv3 double null mutation	2	
BRO1	YPL084W	BCK1-like Resistance to Osmotic shock: Cytoplasmic class E vacuolar protein sorting (VPS) factor that coordinates deubiquitination in the multivesicular body (MVB) pathway by recruiting Doa4p to endosomes	2	
BSC1	YDL037C	Bypass of Stop Codon: Protein of unconfirmed function, similar to cell surface flocculin Muc1p; ORF exhibits genomic organization compatible with a translational readthrough-dependent mode of expression	1	
BSD2	YBR290W	Bypass Sod1p Defects: Heavy metal ion homeostasis protein, facilitates trafficking of Smf1p and Smf2p metal transporters to the vacuole where they are degraded, controls metal ion transport, prevents metal hyperaccumulation, functions in copper detoxification	2	
BST1	YFL025C	Bypass of Sec Thirteen: GPI inositol deacylase of the ER that negatively regulates COPII vesicle formation, prevents production of vesicles with defective subunits, required for proper discrimination between resident ER proteins and Golgi-bound cargo molecules	2	SSS
BUB1	YGR188C	Protein kinase that forms a complex with Mad1p and Bub3p that is crucial in the checkpoint mechanism required to prevent cell cycle progression into anaphase in the presence of spindle damage, associates with centromere DNA via Skp1p	3	
BUB3	YOR026W	Budding Uninhibited by Benzimidazole: Kinetochore checkpoint WD40 repeat protein that localizes to kinetochores during prophase and metaphase, delays anaphase in the presence of unattached kinetochores; forms complexes with Mad1p-Bub1p and with Cdc20p, binds Mad2p and Mad3p	1	
BUD6	YLR319C	BUD site selection: Actin- and formin-interacting protein, involved in actin cable nucleation and polarized cell growth; isolated as bipolar budding mutant; potential Cdc28p substrate	1	
BUD14	YAR014C	BUD site selection: Protein involved in bud-site selection; diploid mutants display a random budding pattern instead of the wild-type bipolar pattern	2	
BUD16	YEL029C	BUD site selection: Protein involved in bud-site selection; diploid mutants display a random budding pattern instead of the wild-type bipolar pattern; has similarity to pyridoxal kinases	1, 2	S; SS*
BUD23	YCR047C	BUD site selection: Protein involved in bud-site selection; diploid mutants display a random budding pattern instead of the wild-type bipolar pattern	2, 3	
BUD25	YER014C-A	BUD site selection: Protein involved in bud-site selection; diploid mutants display a random budding pattern instead of the wild-type bipolar pattern	3, 3	SS
BUD27	YFL023W	BUD site selection: Protein involved in bud-site selection, nutrient signaling, and gene expression controlled by the TOR kinase; diploid mutants display a random budding pattern instead of the wild-type bipolar pattern	2	SSS
BUD32*	YGR262C	BUD site selection: Protein involved in bud-site selection; diploid mutants display a random budding pattern instead of the wild-type bipolar pattern; SGD after 20 & 60 generations, SGD on NaCl	3, 3	
BUR2*	YLR226W	Bypass UAS Requirement: Cyclin for the Sgv1p (Bur1p) protein kinase; Sgv1p and Bur2p comprise a CDK-cyclin complex involved in transcriptional regulation through its phosphorylation of the carboxy-terminal domain of the largest subunit of RNA polymerase II; SGD after 20 generations, MGD after 60 generations, MGD on NaCl	3, 3	
CAF17	YJR122W	CCR4 Associated Factor: Mitochondrial protein that interacts with Ccr4p in the two-hybrid system; 3'-untranslated region contains a putative mRNA localization element common to genes encoding mitochondrial proteins	2, 3	

Appendix B: Zymocin sensitive diploid deletion mutants in *S. cerevisiae*

CAF20	YOR276W	CCR4 Associated Factor: Phosphoprotein of the mRNA cap-binding complex involved in translational control, repressor of cap-dependent translation initiation, competes with eIF4G for binding to eIF4E, Also known as: p20; MGD after 20 & 60 generations, MGD on NaCl	3	
CAP1	YKL007W	Alpha subunit of the capping protein (CP) heterodimer (Cap1p and Cap2p) which binds to the barbed ends of actin filaments preventing further polymerization; localized predominantly to cortical actin patches	3	
CAX4*	YGR036C	CAlmodulin-dependent in cmd one two twenty-siX: Dolichyl pyrophosphate (Dol-P-P) phosphatase with a luminally oriented active site in the ER, cleaves the anhydride linkage in Dol-P-P, required for Dol-P-P-linked oligosaccharide intermediate synthesis and protein N-glycosylation; MGD after 20 & 60 generations, MGD on NaCl	3, 3	
CBP1	YJL209W	Cytochrome B mRNA Processing: Protein required for COB mRNA stability or 5' processing, required for translation of COB mRNAs	3, 2	
CBP2	YHL038C	Protein required for splicing of COB aI5 intron; Cytochrome B pre-mRNA processing protein	3, 2	
CBP3	YPL215W	Protein required for assembly of ubiquinol cytochrome-c reductase complex (cytochrome bc1 complex); involved in cytochrome-c reductase assembly	2, 2	SSS*
CBS1	YDL069C	Cytochrome B Synthesis: translational activator of cytochrome b	2, 3	SS
CBS2	YDR197W	Translational activator of COB mRNA; soluble protein; cytochrome b translational activator	2, 3	
CCC2	YDR270W	copper-transporting P-type ATPase with similarity to human Menkes and Wilsons genes; Cu(2+)-transporting ATPase	2	
CCW12	YLR110C	Covalently linked Cell Wall protein: Cell wall protein, mutants are defective in mating and agglutination, expression is downregulated by alpha-factor	3	
CCW14	YLR390W-A	cell wall mannoprotein, Secretory Stress Response protein	2	
CDC10	YCR002C	Cell Division Cycle: Component of the septin ring of the mother-bud neck that is required for cytokinesis; septins recruit proteins to the neck and can act as a barrier to diffusion at the membrane, and they comprise the 10nm filaments seen with EM	3	
CDC50	YCR094W	Cell Division Cycle: Endosomal protein that regulates cell polarity; similar to Ynr048wp and Lem3p; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	3, 3	
CEM1	YER061C	homology with beta-keto-acyl synthases; Protein homologous to beta-keto-acyl synthase; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2, 3	S, SS
CHC1	YGL206C	Clathrin Heavy Chain: vesicle coat protein; presumed vesicle coat protein	2, 3	SSS
CHD1	YER164W	Chromatin organization modifier, Helicase, and DNA-binding domains: Sole S. cerevisiae member of CHD gene family containing Chromodomain, Helicase domain, and DNA-binding domain; transcriptional regulator, Null mutant is viable, resistant to 6-azauracil	1	
CHL1*	YPL008W	Required for mitotic chromosome segregation, needed for wild-type levels of meiotic recombination and spore viability; kinetochore protein in the DEAH box family	3	
CIK1	YMR198W	Chromosome Instability and Karyogamy: CIK1 is important for proper organiziation of microtubule arrays and establishment of a spindle; is essential for karyogamy; and expression is regulated by KAR4 and mating; spindle pole body associated protein	1	
CIN8	YEL061C	Kinesin motor protein involved in mitotic spindle assembly and chromosome segregation	2	
CIT1	YNR001C	CITrate synthase: Citrate synthase, catalyzes the condensation of acetyl coenzyme A and oxaloacetate to form citrate; the rate-limiting enzyme of the TCA cycle; nuclear encoded mitochondrial protein; Also known as: CS1	1	
CLC1*	YGR167W	Clathrin light chain; MGD after 20 generations, SGD after 60 generations	2, 3	SSS, SSS*
CLN3	YAL040C	role in cell cycle START; involved in G(sub)1 size control; G(sub)1 cyclin	2	
CNM67*	YNL225C	chaotic nuclear migration; predicted mass is 67kDa; Spindle pole body protein involved in nuclear migration	1, 2	S, SSS*
COG1	YGL223C	Component of the conserved oligomeric Golgi complex; interacts with Cog2p; Interacts with Sec35	2	SSS
COG6	YNL041C	Component of the conserved oligomeric Golgi complex; interacts with Cog2p; Sec35 interacting protein; MGD at 20 & 60 generations, MGD on NaCl	1	
COG7	YGL005C	Component of the conserved oligomeric Golgi complex (Cog1p through Cog8p), a cytosolic tethering complex that functions in protein trafficking to mediate fusion of transport vesicles to Golgi compartments	3	
COG8	YML071C	Component of the conserved oligomeric Golgi complex, dependent on RIC1	1	SSS, SSS*
COQ1	YBR003W	Hexaprenyl pyrophosphate synthetase, catalyzes the first step in ubiquinone (coenzyme Q) biosynthesis	2	
COQ2	YNR041C	COenzyme Q: Para hydroxybenzoate: polyprenyl transferase, catalyzes the second step in ubiquinone (coenzyme Q) biosynthesis	3	
COQ4	YDR204W	COenzyme Q: Protein with a role in ubiquinone (Coenzyme Q) biosynthesis, possibly functioning in stabilization of Coq7p; located on the matrix face of the mitochondrial inner membrane; component of a mitochondrial ubiquinone-synthesizing complex	2	
COQ6	YGR255C	COenzyme Q: Putative flavin-dependent monooxygenase, involved in ubiquinone (Coenzyme Q) biosynthesis; located on the matrix side of the mitochondrial inner membrane; MGD after 20 & 60 generations	2, 3	
COX5A	YNL052W	Cytochrome c Oxidase: Subunit Va of cytochrome c oxidase, which is the terminal member of the mitochondrial inner membrane electron transport chain; predominantly expressed during aerobic growth while its isoform Vb (Cox5Bp) is expressed during anaerobic growth	1, 2	
COX6	YHR051W	Cytochrome c Oxidase: Subunit VI of cytochrome c oxidase, which is the terminal member of the mitochondrial inner membrane electron transport chain; expression is regulated by oxygen levels	1, 2	
COX9	YDL067C	Cytochrome c Oxidase: Subunit VIIa of cytochrome c oxidase, which is the terminal member of the mitochondrial inner membrane electron transport chain	1, 3	
COX10	YPL172C	Cytochrome c Oxidase: Heme A:farnesyltransferase, catalyzes the first step in the conversion of protoheme to the heme A prosthetic group required for cytochrome c oxidase activity; human ortholog is associated with mitochondrial disorders	1	
COX11	YPL132W	Cytochrome c Oxidase: Mitochondrial membrane protein required for assembly of active cytochrome c oxidase, probably involved in insertion of Cu(B) and magnesium	1, 2	
COX14	YML129C	Cytochrome c Oxidase: Mitochondrial membrane protein, required for assembly of cytochrome c oxidase	2, 2	
COX15	YER141W	Cytochrome c Oxidase: Protein required for the hydroxylation of heme O to form heme A, which is an essential prosthetic group for cytochrome c oxidase; MGD after 20 generations, SGD after 60 enerations, MGD on Nacl	1, 3	
COX16	YJL160C	Mitochondrial inner membrane protein, required for assembly of cytochrome c oxidase	2, 2	
COX17	YLL009C	Cytochrome c Oxidase: Copper metallochaperone that shuttles copper from the cytosol to the mitochondrial intermembrane space for delivery to cytochrome c oxidase	3, 2	
COX18	YGR062C	Cytochrome c Oxidase: Mitochondrial inner membrane protein, required for export of the Cox2p C terminus from the mitochondrial matrix to the intermembrane space during its assembly into cytochrome c oxidase; similar to Oxa2p of N.crassa	2, 2	
COX19	YLL018C-A	Cytochrome c Oxidase: Protein required for cytochrome c oxidase assembly, located in the cytosol and mitochondrial intermembrane space; putative copper metallochaperone that delivers copper to cytochrome c oxidase	3, 2	

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COX20	YDR231C	Cytochrome c Oxidase: Mitochondrial inner membrane protein, required for proteolytic processing of Cox2p and its assembly into cytochrome c oxidase	1, 2	S; SS*
COX23	YHR116W	Cytochrome Oxidase: Mitochondrial intermembrane space protein that functions in mitochondrial copper homeostasis, essential for functional cytochrome oxidase expression; homologous to Cox17p; MGD after 20 & 60 generations	2, 2	
CSE1	YNR010W	Chromosome Segregation: Component of the Med9/10 module, which is a subcomplex within the RNA polymerase II Mediator complex; required for regulation of RNA polymerase II activity	2	
CSF1	YLR087C	Cold Sensitive for Fermentation: Protein required for fermentation at low temperature	3	
CSM1	YCR086W	Chromosome Segregation in Meiosis: Protein that forms a complex with Lrs4p, located in the nucleolus; Lrs4p-Csm1p heterodimer binds to Mam1p at kinetochores during meiosis I to mediate accurate chromosome segregation, may be involved in premeiotic DNA replication	1	
CSM3	YMR048W	Chromosome Segregation in Meiosis: Protein required for accurate chromosome segregation during meiosis; MGD after 20 & 60 generations, MGD on NaCl	1	
CTF3	YLR381W	Outer kinetochore protein that forms a complex with Mcm16p and Mcm22p; may bind the kinetochore to spindle microtubules	2	
CTF4*	YPR135W	Chromatin-associated protein, required for sister chromatid cohesion; interacts with DNA polymerase alpha (Pol1p) and may link DNA synthesis to sister chromatid cohesion	3	
CTF8*	YHR191C	Subunit of a complex with Ctf18p that shares some subunits with Replication Factor C and is required for sister chromatid cohesion	3	
CTF18	YMR078C	Chromosome Transmission Fidelity: Subunit of a complex with Ctf8p that shares some subunits with Replication Factor C and is required for sister chromatid cohesion; may have overlapping functions with Rad24p in the DNA damage replication checkpoint	3	
CTF19	YPL018W	Outer kinetochore protein, required for accurate mitotic chromosome segregation; forms a complex with Mcm21p and Okp1p that binds to centromeres via the CBF3 complex	1	SSS, SS*
CTP1	YBR291C	Citrate Transport Protein: Mitochondrial inner membrane citrate transporter, member of the mitochondrial carrier family	2	
CUP5	YEL027W	Proteolipid subunit of the vacuolar H(+)-ATPase V0 sector (subunit c; dicyclohexylcarbodiimide binding subunit); required for vacuolar acidification and important for copper and iron metal ion homeostasis	2	
CUP9	YPL177C	Homeodomain-containing transcriptional repressor of PTR2, which encodes a major peptide transporter; imported peptides activate ubiquitin-dependent proteolysis, resulting in degradation of Cup9p and de-repression of PTR2 transcription	1	SS, SS*
CWH36*	YCL007C	Dubious open reading frame that overlaps YCL005W-A (87%); mutations in YCL007C were thought to confer sensitivity to calcofluor white, but this phenotype was later shown to be due to the defect in YCL005W-A	3	
CWH41	YGL027C	Processing alpha glucosidase I, involved in assembly of cell wall beta 1,6 glucan and asparagine-linked protein glycosylation; ER type II integral membrane N-glycoprotein; disruption leads to a K1 killer toxin-resistant phenotype	1	SS, SS*
CWH43	YCR017C	Putative sensor/transporter protein involved in cell wall biogenesis; contains 14-16 transmembrane segments and several putative glycosylation and phosphorylation sites; null mutation is synthetically lethal with pkc1 deletion	3	
CYC2	YOR037W	Mitochondrial protein required for normal abundance of mitochondrial cytochrome c (Cyc1p) and for mitochondrial osmotic stability; may be involved in regulating the activity of cytochrome c heme lyase (Cyc3p); potential Cdc28p substrate	2	S*
CYC3	YAL039C	Cytochrome c heme lyase (holocytochrome c synthase), attaches heme to apo-Cyc1p in the mitochondrial intermembrane space; human ortholog may have a role in microphthalmia with linear skin defects	2, 2	
CYC8	YBR112C	General transcriptional co-repressor, acts together with Tup1p; also acts as part of a transcriptional co-activator complex that recruits the SWI/SNF and SAGA complexes to promoters	3	SSS
CYT1	YOR065W	Cytochrome c1	1, 2	SS*
DAL81	YIR023W	Degradation of Allantoin: Positive regulator of genes in multiple nitrogen degradation pathways; contains DNA binding domain but does not appear to bind the dodecanucleotide sequence present in the promoter region of many genes involved in allantoin catabolism, MGD after 20 generations, SGD after 60 generations, SGD on NaCl	2	
DBF2*	YGR092W	DumbBell Forming: Ser/Thr kinase involved in transcription and stress response; functions as part of a network of genes in exit from mitosis; localization is cell cycle regulated; activated by Cdc15p during the exit from mitosis	3	
DCC1*	YCL016C	Subunit of a complex with Ctf8p and Ctf18p that shares some components with Replication Factor C, required for sister chromatid cohesion	3	
DCS1	YLR270W	DeCapping Scavenger: Non-essential hydrolase involved in mRNA decapping, may function in a feedback mechanism to regulate deadenylation, contains pyrophosphatase activity and a HIT (histidine triad) motif; interacts with neutral trehalase Nth1p	1, 2	S*
DEF1*	YKL054C	RNAPII DEgradation Factor: forms a complex with Rad26p in chromatin, enables ubiquitination and proteolysis of RNAPII	2	
DEG1*	YFL001W	DEpressed Growth rate: Non-essential tRNA:pseudouridine synthase, introduces pseudouridines at position 38 or 39 in tRNA, important for maintenance of translation efficiency and normal cell growth, localizes to both the nucleus and cytoplasm; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	3	
DEM1	YBR163W	Protein of unknown function, shows similarity to RNA-processing protein Pta1p	3	SSS
DER1	YBR201W	Degradation in the Endoplasmic Reticulum: Endoplasmic reticulum membrane protein, required for the protein degradation process associated with the ER, involved in the retrograde transport of misfolded or unassembled proteins	1	SS*
DFG16	YOR030W	Defective for Filamentous Growth: Probable multiple transmembrane protein, involved in invasive growth upon nitrogen starvation	3	
DHH1*	YDL160C	Cytoplasmic DExD/H-box helicase, stimulates mRNA decapping, coordinates distinct steps in mRNA function and decay, interacts with both the decapping and deadenylase complexes, may have a role in mRNA export and translation	3, 3	
DIA2	YOR080W	Digs Into Agar: Protein of unknown function, involved in invasive and pseudohyphal growth	1	
DIA4*	YHR011W	Digs Into Agar: Probable mitochondrial seryl-tRNA synthetase, mutant displays increased invasive and pseudohyphal growth; SGD after 20 generations, MGD after 60 generations, MGD on NaCl	3	
DID4	YKL002W	Doa4-Independent Degradation: Class E Vps protein of the ESCRT-III complex, required for sorting of integral membrane proteins into lumenal vesicles of multivesicular bodies, and for delivery of newly synthesized vacuolar enzymes to the vacuole, involved in endocytosis; SGD after 20 & 60 generations, MGD on NaCl	2, 2	
DIE2	YGR227W	Dolichyl-phosphoglucose-dependent glucosyltransferase of the ER, functions in the dolichol pathway that synthesizes the dolichol-linked oligosaccharide precursor for N-linked protein glycosylation, has a role in regulation of ITR1 and INO1	2	
DOA4	YDR069C	Ubiquitin hydrolase, required for recycling ubiquitin from proteasome-bound ubiquitinated intermediates, acts at the late endosome/prevacuolar compartment to recover ubiquitin from ubiquitinated membrane proteins en route to the vacuole	3	
DOC1*	YGL240W	Destruction Of Cyclin B: Processivity factor required for the ubiquitination activity of the anaphase promoting complex (APC), mediates the activity of the APC by contributing to substrate recognition; involved in cyclin proteolysis	2, 3	SSS

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DRS2	YAL026C	Integral membrane Ca(2+)-ATPase, potential aminophospholipid translocase required to form a specific class of secretory vesicles that accumulate upon actin cytoskeleton disruption; mutation affects maturation of the 18S rRNA	2	
DSS1	YMR287C	3'-5' exonuclease complex component: RNase, component of the mitochondrial degradosome along with the ATP-dependent RNA helicase Suv3p; the degradosome associates with the ribosome and mediates turnover of aberrant or unprocessed RNAs	1, 2	
DUR1,2	YBR208C	Urea amidolyase, contains both urea carboxylase and allophanate hydrolase activities, degrades urea to CO2 and NH3; expression sensitive to nitrogen catabolite repression and induced by allophanate, an intermediate in allantoin degradation	1	S, SS*
EAF3	YPR023C	Esa1p-Associated Factor: Esa1p-associated factor, nonessential component of the NuA4 acetyltransferase complex, homologous to Drosophila dosage compensation protein MSL3; SGD after 20 & 60 generations, SGD on NaCl	2	
EAF7	YNL136W	Esa1-Associated Factor: Subunit of the NuA4 histone acetyltransferase complex	2	
EBS1	YDR206W	EST1-like Bcy1 Suppressor: Protein of unknown function, contains a putative RNA recognition motif, deletion results in short telomeres; similar to Est1p, may be partially redundant with Est1p for telomere maintenance	2	
ECM37	YIL148W	ExtraCellular Mutant: Tn3 insertion mutant demonstrates hypersensitivity to the cell surface polymer perturbing agent calcofluor white; MGD after 20 generations, MGD on Nacl	1	
ECM39	YNR030W	ExtraCellular Mutan: Alpha-1,6-mannosyltransferase localized to the ER; responsible for the addition of the alpha-1,6 mannose to dolichol-linked Man7GlcNAc2, acts in the dolichol pathway for N-glycosylation; MGD at 20 & 60 generations, SGD on NaCl	1	
EDE1	YBL047C	Key endocytic protein involved in a network of interactions with other endocytic proteins, binds membranes in a ubiquitin-dependent manner, may also bind ubiquitinated membrane-associated proteins	1	SSS, SS*
ELM1*	YKL048C	ELongated Morphology: Serine/threonine protein kinase that regulates cellular morphogenesis, septin behavior, and cytokinesis; required for the regulation of other kinases; forms part of the bud neck ring	3	
EMI1	YDR512C	Early Meiotic Induction: Non-essential protein of unknown function required for transcriptional induction of the early meiotic-specific transcription factor IME1, also required for sporulation; MGD after 20 & 60 generations, MGD on NaCl	3, 2, 3	SSS
EMI2	YDR516C	Early Meiotic Induction: Non-essential protein of unknown function required for transcriptional induction of the early meiotic-specific transcription factor IME1, also required for sporulation	2	
END3	YNL084C	ENDocytosis defective: EH domain-containing protein involved in endocytosis, actin cytoskeletal organization and cell wall morphogenesis; forms a complex with Sla1p and Pan1p	2	
ENT5	YDR153C	Epsin N-Terminal homology: Protein containing an N-terminal epsin-like domain involved in clathrin recruitment and traffic between the Golgi and endosomes; associates with the clathrin adaptor Gga2p, clathrin adaptor complex AP-1, and clathrin	1	SS, SS*
EPS1	YIL005W	Pdi1p (protein disulfide isomerase)-related protein involved in endoplasmic reticulum retention of resident ER proteins	2	
ERG3*	YLR056W	ERGosterol biosynthesis: C-5 sterol desaturase, catalyzes the introduction of a C-5(6) double bond into episterol, a precursor in ergosterol biosynthesis; mutants are viable, but cannot grow on non-fermentable carbon sources	3	
ERG4	YGL012W	C-24(28) sterol reductase, catalyzes the final step in ergosterol biosynthesis; mutants are viable, but lack ergosterol	2	SS
ERG6	YMP008C	Delta(24)-sterol C-methyltransferase, converts zymosterol to fecosterol in the ergosterol biosynthetic pathway by methylating position C-24	2	
ERG24	YNL280C	C-14 sterol reductase, acts in ergosterol biosynthesis; mutants accumulate the abnormal sterol ignosterol (ergosta-8,14 dienol), and are viable under anaerobic growth conditions but inviable on rich medium under aerobic conditions	1, 3	SSS
ERV14	YGL054C	Protein localized to COPII-coated vesicles, involved in vesicle formation and incorporation of specific secretory cargo; required for the delivery of bud-site selection protein Axl2p to cell surface; related to Drosophila cornichon	2	
EST1*	YLR233C	Ever Shorter Telomeres: TLC1 RNA-associated factor involved in telomere length regulation as the recruitment subunit of the telomerase holoenzyme, has a possible role in activating Est2p-TLC1-RNA bound to the telomere	2	
EST2	YLR318W	Reverse transcriptase subunit of the telomerase holoenzyme, essential for telomerase core catalytic activity, involved in other aspects of telomerase assembly and function	1	
ETR1	YBR026C	2-Enoyl Thioester Reductase: 2-enoyl thioester reductase, member of the medium chain dehydrogenase/reductase family; localized to in mitochondria, where it has a probable role in fatty acid synthesis; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1, 3	
EUG1	YDR518W	ER protein Unnecessary for Growth under standard laboratory conditions: Protein disulfide isomerase of the endoplasmic reticulum lumen, function overlaps with that of Pdi1p; may interact with nascent polypeptides in the ER	2, 3	SSS
FAB1*	YFR019W	Forms aploid and binucleate cells: 1-phosphatidylinositol-3-phosphate 5-kinase; vacuolar membrane kinase that generates phosphatidylinositol (3,5)P2, which is involved in vacuolar sorting and homeostasis	3, 3	
FAR7	YFR008W	Protein involved in G1 cell cycle arrest in response to pheromone, in a pathway different from the Far1p-dependent pathway; interacts with Far3p, Far8p, Far9p, Far10p, and Far11p	2	
FAR10	YLR238W	Protein involved in G1 cell cycle arrest in response to pheromone, in a pathway different from the Far1p-dependent pathway; interacts with Far3p, Far7p, Far8p, Far9p, and Far11p; potential Cdc28p substrate	2	
FBP26	YJL155C	Fructose-2,6-bisphosphatase, required for glucose metabolism Also known as: FBPase-2	2	
FEN2	YCR028C	Plasma membrane H+-pantothenate symporter; confers sensitivity to the antifungal agent fenpropimorph	2	
FIG4	YNL325C	Factor-Induced Gene: Protein required for efficient mating, member of a family of eukaryotic proteins that contain a domain homologous to Sac1p	2	
FKS1	YLR342W	FK506 Sensitivity: Catalytic subunit of 1,3-beta-D-glucan synthase, functionally redundant with alternate catalytic subunit Gsc2p; binds to regulatory subunit Rho1p; involved in cell wall synthesis and maintenance; localizes to sites of cell wall remodeling	3	
FMC1	YIL098C	Formation of Mitochondrial Complexes: Mitochondrial matrix protein, required for assembly or stability at high temperature of the F1 sector of mitochondrial F1F0 ATP synthase	2	
FMP38	YOR205C	Found in Mitochondrial Proteome: The authentic, non-tagged protein was localized to the mitochondria	2, 2	
FMP53	YLR201C	Found in Mitochondrial Proteome: The authentic, non-tagged protein was localized to the mitochondria	2, 2	
FPR2	YDR519W	FKBP Proline Rotamase: Membrane-bound peptidyl-prolyl cis-trans isomerase (PPIase), binds to the drugs FK506 and rapamycin; expression pattern suggests possible involvement in ER protein trafficking, Also known as: FKBP13	2, 3	SSS
FTR1	YER145C	Fe Transporter: High affinity iron permease involved in the transport of iron across the plasma membrane; forms complex with Fet3p; expression is regulated by iron	1	
FUM1	YPL262W	Fumarase, converts fumaric acid to L-malic acid in the TCA cycle; cytosolic and mitochondrial localization determined by the N-terminal mitochondrial targeting sequence and protein conformation	1, 3	SSS, SSS*
FYV4	YHR059W	Protein of unknown function, required for survival upon exposure to K1 killer toxin	3	

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FYV6	YNL133C	Protein of unknown function, required for survival upon exposure to K1 killer toxin; proposed to regulate double-strand break repair via non-homologous end-joining	2, 3	SSS
FYV8	YGR196C	Function required for Yeast Viability 1: Protein of unknown function, required for survival upon exposure to K1 killer toxin	2	
FYV10	YIL097W	Function required for Yeast Viability: Protein of unknown function, required for survival upon exposure to K1 killer toxin; involved in proteasome-dependent catabolite inactivation of fructose-1,6-bisphosphatase; contains CTLH domain	2	
FZO1	YBR179C	FuZzy Onions homolog: Mitochondrial integral membrane protein involved in mitochondrial fusion and maintenance of the mitochondrial genome; contains N-terminal GTPase domain	1, 2	SS*
GAL1	YBR020W	GALactose metabolism: Galactokinase, phosphorylates alpha-D-galactose to alpha-D-galactose-1-phosphate in the first step of galactose catabolism; expression regulated by Gal4p; MGD after 20 & 60 generations, MGD on NaCl	2	
GAL11	YOL051W	GALactose metabolism: Component of the Mediator complex; interacts with RNA polymerase II and the general transcription factors to form the RNA polymerase II holoenzyme; affects transcription by acting as target of activators and repressors	2, 3	SSS
GAS1	YMR307W	Glycophospholipid-Anchored Surface protein: Beta-1,3-glucanosyltransferase, required for cell wall assembly; localizes to the cell surface via a glycosylphosphatidylinositol (GPI) anchor	3	
GCS1	YDL226C	ADP-ribosylation factor GTPase activating protein (ARF GAP), involved in ER-Golgi transport; shares functional similarity with Glo3p	1	S, SS*
GCV3	YAL044C	GlyCine cleaVage: H subunit of the mitochondrial glycine decarboxylase complex, required for the catabolism of glycine to 5,10-methylene-THF; expression is regulated by levels of levels of 5,10-methylene-THF in the cytoplasm; MGD after 20 & 60 generations	2, 2	
GEF1	YJR040W	Glycerol Ethanol, Ferric requiring: Chloride channel localized to late- or post-Golgi vesicles, involved in iron metabolism; highly homologous to voltage-gated chloride channels in vertebrates	2	
GGA2	YHR108W	Golgi-localized protein with homology to gamma-adaptin, interacts with and regulates Arf1p and Arf2p in a GTP-dependent manner in order to facilitate traffic through the late Golgi	3	
GGC1	YDL198C	Mitochondrial GTP/GDP transporter, essential for mitochondrial genome maintenance; has a role in mitochondrial iron transport; member of the mitochondrial carrier family; (putative) mitochondrial carrier protein	3	
GIN4	YDR507C	Growth Inhibitory: Protein kinase involved in bud growth and assembly of the septin ring, proposed to have kinase-dependent and kinase-independent activities; undergoes autophosphorylation; similar to Kcc4p and Hsl1p	2, 3	SSS; SS*
GLC8	YMR311C	Regulatory subunit of protein phosphatase 1 (Glc7p), involved in glycogen metabolism and chromosome segregation; proposed to regulate Glc7p activity via conformational alteration; ortholog of the mammalian protein phosphatase inhibitor 2	1	SSS, SS*
GLO3*	YER122C	GLyOxalase: ADP-ribosylation factor GTPase activating protein (ARF GAP), involved in ER-Golgi transport; shares functional similarity with Gcs1p	3, 3	SSS
GON7*	YJL184W	Protein of unknown function, proposed to be involved in the transfer of mannosylphosphate groups onto N-linked oligosaccharides; also proposed to be involved in responding to osmotic stress	2, 3	SSS
GOS1*	YHL0341C	SNARE protein with a C-terminal membrane anchor; Golgi SNARE protein	3	
GPB1	YOR371C	Proposed beta subunit of the heterotrimeric G protein that interacts with the receptor Grp1p, has signaling role in response to nutrients; involved in regulation of pseudohyphal growth through cAMP levels; homolog of Gpb2p	1	SS, SS*
GPB2	YAL056W	Proposed beta subunit of the heterotrimeric G protein that interacts with the receptor Grp1p, has signaling role in response to nutrients; involved in regulation of pseudohyphal growth through cAMP levels; homolog of Gpb1p	2	
GPD2	YOL059W	Involved in glycerol production via conversion of glyerol-3-phosphate and NAD+ to glycerol phosphate and NADH; Glycerol-3-phosphate dehydrogenase (NAD+)	1	
GPX2	YBR244W	Phospholipid hydroperoxide glutathione peroxidase induced by glucose starvation that protects cells from phospholipid hydroperoxides and nonphospholipid peroxides during oxidative stress	3	
GRD19	YOR357C	Golgi Retention Deficient: Sorting nexin required to maintain late-Golgi resident enzymes in their proper location by recycling molecules from the prevacuolar compartment; contains a PX domain and sequence similarity to human Snx3p	1	SS, SS*
GRE3	YHR104W	Aldose reductase involved in methylglyoxal, d-xylose and arabinose metabolism; stress induced (osmotic, ionic, oxidative, heat shock, starvation and heavy metals); regulated by the HOG pathway	1	
GRR1*	YJR090C	F-box protein component of the SCF ubiquitin-ligase complex, required for Cln1p and Cln2p degradation; involved in carbon catabolite repression, glucose-dependent divalent cation transport, high-affinity glucose transport, and morphogenesis	3, 3	SSS
GSG1	YDR108W	Subunit of TRAPP (transport protein particle), a multi-subunit complex involved in targeting and/or fusion of ER-to-Golgi transport vesicles with their acceptor compartment; protein has late meiotic role, following DNA replication	2	
GSH2	YOL049W	Glutathione synthetase, catalyzes the ATP-dependent synthesis of glutathione (GSH) from gamma-glutamylcysteine and glycine; induced by oxidative stress and heat shock	1	
GUP1*	YGL084C	Glycerol Uptake: Multimembrane-spanning protein and putative glycerol transporter that is essential for proton symport of glycerol; Gup2p homolog	2	SSS
GVP36	YIL041W	Golgi-vesicle protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm	2	
HAL9	YOL089C	HALotolerance: Putative transcription factor containing a zinc finger; overexpression increases salt tolerance through increased expression of the ENA1 (Na+/Li+ extrusion pump) gene while gene disruption decreases both salt tolerance and ENA1 expression	1	SSS, SSS*
HAP2	YGL237C	Heme Activator Protein: Subunit of the heme-activated, glucose-repressed Hap2p/3p/4p/5p CCAAT-binding complex, a transcriptional activator and global regulator of respiratory gene expression; contains sequences sufficient for both complex assembly and DNA binding	2, 2	
HAP4	YKL109W	Heme Activator Protein: Subunit of the heme-activated, glucose-repressed Hap2p/3p/4p/5p CCAAT-binding complex, a transcriptional activator and global regulator of respiratory gene expression; provides the principal activation function of the complex; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1, 2	
HDA1	YNL021W	Histone DeAcetylase: Putative catalytic subunit of a class II histone deacetylase complex that also contains Hda2p and Hda3p; Hda1p interacts with the Hda2p-Hda3p subcomplex to form an active tetramer; deletion increases histone H2B, H3 and H4 acetylation	2	
HEK2	YBL032W	RNA binding protein with similarity to hnRNP-K that localizes to the cytoplasm and to subtelomeric DNA; required for the proper localization of ASH1 mRNA; involved in the regulation of telomere position effect and telomere length	2	
HEX3	YDL013W	HEXose: Ring finger protein involved in the DNA damage response with possible recombination role; genetically identified by synthetic lethality with SGS1 (DNA helicase) and TOP3 (DNA topoisomerase); sporulation role; interacts with Slx8p and Lin1p	1	
HFI1*	YPL254W	Adaptor protein required for structural integrity of the SAGA complex, a histone acetyltransferase-coactivator complex that is involved in global regulation of gene expression through acetylation and transcription functions	3, 3	SSS, SSS*

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HFM1	YGL251C	Helicase Family Member: Meoisis specific DNA helicase involved in the conversion of double-standed breaks to later recombination intermediates and in crossover control; catalyzes the unwinding of Holliday junctions; has ssDNA and dsDNA stimulated ATPase activity	2	
HIS7	YBR248C	HIStidine requiring: Imidazole glycerol phosphate synthase (glutamine amidotransferase:cyclase), catalyzes the fifth and sixth steps of histidine biosynthesis and also produces 5-aminoimidazole-4-carboxamide	1	S*
HOC1	YJR075W	Homologous to OCh1: Alpha-1,6-mannosyltransferase involved in cell wall mannan biosynthesis; subunit of a Golgi-localized complex that also contains Anp1p, Mnn9p, Mnn11p, and Mnn10p; identified as a suppressor of a cell lysis sensitive pkc1-371 allele	3	
HOG1	YLR113W	High Osmolarity Glycerol response: Mitogen-activated protein kinase involved in osmoregulation via three independent osmosensors; mediates the recruitment and activation of RNA Pol II at Hot1p-dependent promoters; localization regulated by Ptp2p and Ptp3p	3	
HOM2	YDR158W	HOMoserine requiring: Aspartic beta semi-aldehyde dehydrogenase, catalyzes the second step in the common pathway for methionine and threonine biosynthesis; expression regulated by Gcn4p and the general control of amino acid synthesis	2	
HOM3	YER052C	HOMoserine requiring: Aspartate kinase (L-aspartate 4-P-transferase); cytoplasmic enzyme that catalyzes the first step in the common pathway for methionine and threonine biosynthesis; expression regulated by Gcn4p and the general control of amino acid synthesis; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2	SS
HOM6	YJR139C	HOMoserine requiring: Homoserine dehydrogenase (L-homoserine:NADP oxidoreductase), dimeric enzyme that catalyzes the third step in the common pathway for methionine and threonine biosynthesis; enzyme has nucleotide-binding, dimerization and catalytic regions	2	
HSP31	YDR533C	Heat-Shock Protein: Possible chaperone and cysteine protease with similarity to E. coli Hsp31 and S. cerevisiae Hsp32p, Hsp33p, and Sno4p; member of the DJ-1/ThiJ/Pfp1 superfamily, which includes human DJ-1 involved in Parkinson's disease; exists as a dimer; MGD after 20 & 60 generations, MGD on NaCl	2, 3	
HTD2	YHR067W	Mitochondrial 3-hydroxyacyl-thioester dehydratase involved in fatty acid biosynthesis, required for respiratory growth and for normal mitochondrial morphology	2, 2	
HTL1*	YCR020W-B	High-Temperature Lethal: Subunit of the RSC chromatin remodeling complex, a multisubunit complex that functions in transcriptional regulation, chromosome stability and establishing sister chromatid cohesion	2	
HTZ1	YOL012C	Histone variant H2AZ, exchanged for histone H2A in nucleosomes by the SWR1 complex; involved in transcriptional regulation through prevention of the spread of silent heterochromatin	2	
HUR1	YGL168W	HydroxyUrea Resistance: Protein required for hydroxyurea resistance; has a possible role in DNA replication	3	
HXT4	YHR092C	High-affinity glucose transporter of the major facilitator superfamily, expression is induced by low levels of glucose and repressed by high levels of glucose	3	
ICL2	YPR006C	2-methylisocitrate lyase of the mitochondrial matrix, functions in the methylcitrate cycle to catalyze the conversion of 2-methylisocitrate to succinate and pyruvate; ICL2 transcription is repressed by glucose and induced by ethanol	1	S*
IDH2	YOR136W	Isocitrate DeHydrogenase: Subunit of mitochondrial NAD(+)-dependent isocitrate dehydrogenase, which catalyzes the oxidation of isocitrate to alpha-ketoglutarate in the TCA cycle	2	
IES6	YEL044W	Ino Eighty Subunit: Protein that associates with the INO80 chromatin remodeling complex under low-salt conditions	2	
IFM1	YOL023W	Mitochondrial translation initiation factor 2	2, 2	SSS*
ILM1	YJR118C	Protein of unknown function	2	
ILV1	YER086W	Threonine deaminase, catalyzes the first step in isoleucine biosynthesis; expression is under general amino acid control; ILV1 locus exhibits highly positioned nucleosomes whose organization is independent of known ILV1 regulation	2	
IMG1	YCR047C	Integrity of Mitochondrial Genome: Mitochondrial ribosomal protein of the small subunit, required for respiration and for maintenance of the mitochondrial genome; SGD after 20 & 60 generations, MGD on NaCl	1, 3	
IMG2*	YCR071C	Integrity of Mitochondrial Genome: Mitochondrial ribosomal protein of the small subunit	1, 3	
IMP2	YMR035W	Inner Membrane Protease: Catalytic subunit of the mitochondrial inner membrane peptidase complex, required for maturation of mitochondrial proteins of the intermembrane space; complex contains Imp1p and Imp2p (both catalytic subunits), and Som1p	1, 2	SS*
IMP2'	YIL154C	Transcriptional activator involved in maintenance of ion homeostasis and protection against DNA damage caused by bleomycin and other oxidants, contains a C-terminal leucine-rich repeat; MGD after 20 generations, SGD after 60 generations, SGD on NaCl	2	
IPK1	YDR315C	Inositol 1,3,4,5,6-pentakisphosphate 2-kinase, nuclear protein required for synthesis of 1,2,3,4,5,6-hexakisphosphate (phytate), which is integral to cell function; has 2 motifs conserved in other fungi; ipk1 gle1 double mutant is inviable	2	
IRA2	YOL081W	GTPase-activating protein that negatively regulates RAS by converting it from the GTP- to the GDP-bound inactive form, required for reducing cAMP levels under nutrient limiting conditions, has similarity to Ira1p and human neurofibromin	3	
IRS4	YKR019C	Increased rDNA Silencing: Protein involved in regulation of phosphatidylinositol 4,5-bisphosphate concentrations; Irs4p and Tax4p bind and activate the phosphatase Inp51p; mutation confers an increase in rDNA silencing; MGD after 20 generations, SGD after 60 generations, SGD on NaCl	3	
ISA1	YLL027W	Iron Sulfur Assembly: Mitochondrial matrix protein involved in biogenesis of the iron-sulfur (Fe/S) cluster of Fe/S proteins, isa1 deletion causes loss of mitochondrial DNA and respiratory deficiency; depletion reduces growth on nonfermentable carbon sources	3, 3	
ISA2	YPR067W	Iron Sulfur Assembly: Protein required for maturation of mitochondrial and cytosolic Fe/S proteins, localizes to the mitochondrial intermembrane space, overexpression of ISA2 suppresses grx5 mutations; MGD after 20 generations, SGD after 60 generations, SGD on NaCl	2, 3	SSS
ISM1	YPL040C	Mitochondrial isoleucyl-tRNA synthetase, null mutant is deficient in respiratory growth	2, 2	SS*
ISR1	YPR106W	Inhibition of Staurosporine Resistance: Predicted protein kinase, overexpression causes sensitivity to staurosporine, which is a potent inhibitor of protein kinase C	1	S, SS*
ITC1	YGL133W	Component of the ATP-dependent Isw2p-Itc1p chromatin remodeling complex, required for repression of a-specific genes, repression of early meiotic genes during mitotic growth, and repression of INO1:	2	
IXR1	YKL032C	Intrastrand cross (X)-link Recognition: Protein that binds DNA containing intrastrand cross-links formed by cisplatin, contains two HMG (high mobility group box) domains, which confer the ability to bend cisplatin-modified DNA; mediates aerobic transcriptional repression of COX5b	3	
JID1	YPR061C	Probable Hsp40p co-chaperone, has a DnaJ-like domain and appears to be involved in ER-associated degradation of misfolded proteins containing a tightly folded cytoplasmic domain; inhibits replication of Brome mosaic virus in S. cerevisiae	2	
JIP3	YLR331C	ORF, Dubious: Jumonji Interacting Protein	1	
JNM1	YMR294W	Just Nuclear Migration: Component of the yeast dynactin complex, consisting of Nip100p, Jnm1p, and Arp1p; required for proper nuclear migration and spindle partitioning during mitotic anaphase B	2	
KAP123	YER110C	KAryoPherin: Karyopherin beta, mediates nuclear import of ribosomal proteins prior to assembly into ribosomes and import of histones H3 and H4; localizes to the nuclear pore, nucleus, and cytoplasm; exhibits genetic interactions with RAI1; MGD after 20 & 60 generations, MGD on NaCl	1	

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KAR4	YCL055W	Transcription factor required for induction of KAR3 and CIK1 during mating, also required during meiosis; exists in two forms, a slower-migrating form more abundant during vegetative growth and a faster-migrating form induced by pheromone	1	S, SS*
KGD1	YIL125W	alpha-KetoGlutarate Dehydrogenase: Component of the mitochondrial alpha-ketoglutarate dehydrogenase complex, which catalyzes a key step in the tricarboxylic acid (TCA) cycle, the oxidative decarboxylation of alpha-ketoglutarate to form succinyl-CoA	1, 2	SSS
KHA1	YJL094C	Putative K+/H+ antiporter; MGD after 60 generations, SGD on NaCl	2	
KRE1	YNL322C	Killer toxin Resistant: Cell wall glycoprotein involved in beta-glucan assembly; serves as a K1 killer toxin membrane receptor	2	
KRE2	YDR483W	Killer toxin Resistant: Alpha1,2-mannosyltransferase of the Golgi involved in protein mannosylation; SGD after 20 & 60 generations	2	SSS
LAS21	YJL062W	Integral plasma membrane protein involved in the synthesis of the glycosylphosphatidylinositol (GPI) core structure; mutations affect cell wall integrity	2	
LAT1	YNL071W	Dihydrolipoamide acetyltransferase component (E2) of pyruvate dehydrogenase complex, which catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA, MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2	
LCB5	YLR260W	Long-Chain Base: Minor sphingoid long-chain base kinase, paralog of Lcb4p responsible for few percent of the total activity, possibly involved in synthesis of long-chain base phosphates, which function as signaling molecules	2, 2	S*
LEM3	YNL323W	Ligand-Effect Modulator: Membrane protein of the plasma membrane and ER, involved in translocation of phospholipids and alkylphosphocholine drugs across the plasma membrane	2	
LHS1*	YKL073W	Molecular chaperone of the endoplasmic reticulum lumen, involved in polypeptide translocation and folding; member of the Hsp70 family; localizes to the lumen of the ER; regulated by the unfolded protein response pathway	3	
LIP2	YLR239C	LIPoyl ligase: Lipoyl ligase, involved in the modification of mitochondrial enzymes by the attachment of lipoic acid groups; SGD after 20 generations, MGD after 60 generations, MGD on NaCl	2, 2	SS*
LIP5*	YOR196C	LIPoic acid: Protein involved in biosynthesis of the coenzyme lipoic acid, has similarity to E. coli lipoic acid synthase	1, 2	SS*
LPD1	YFL018C	LiPoamide Dehydrogenase: Dihydrolipoamide dehydrogenase, the lipoamide dehydrogenase component (E3) of the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase multi-enzyme complexes; MGD after 20 generations, MGD on NaCl	2, 2	S, SS
LRE1	YCL051W	Laminarase Resistance: Protein involved in control of cell wall structure and stress response; inhibits Cbk1p protein kinase activity; overproduction confers resistance to cell-wall degrading enzymes	1	S, S*
LST4	YKL176C	Protein possibly involved in a post-Golgi secretory pathway; required for the transport of nitrogen-regulated amino acid permease Gap1p from the Golgi to the cell surface	3	
LST7	YGR057C	Protein possibly involved in a post-Golgi secretory pathway; required for the transport of nitrogen-regulated amino acid permease Gap1p from the Golgi to the cell surface	3	
MAC1	YMR021C	Copper-sensing transcription factor involved in regulation of genes required for high affinity copper transport; SGD after 20 & 60 generations,MGD on NaCl	3, 3	SSS
MAK3	YPR051W	MAintenance of Killer: Catalytic subunit of N-terminal acetyltransferase of the NatC type; required for replication of dsRNA virus	1	SS, SS*
MAM3	YOL060C	Protein required for normal mitochondrial morphology, has similarity to hemolysins	1	
MAP1*	YLR244C	Methionine AminoPeptidase: Methionine aminopeptidase, catalyzes the cotranslational removal of N-terminal methionine from nascent polypeptides; function is partially redundant with that of Map2p	2, 2	S, SSS*
MBF1	YOR298C-A	Multiprotein Bridging Factor: Transcriptional coactivator that bridges the DNA-binding region of Gcn4p and TATA-binding protein Spt15p; suppressor of frameshift mutations	1	
MBP1*	YDL056W	MluI-box Binding Protein: Transcription factor involved in regulation of cell cycle progression from G1 to S phase, forms a complex with Swi6p that binds to MluI cell cycle box regulatory element in promoters of DNA synthesis genes; MGD after 20 generations, SGD after 60 generations, MGD in NaCl	2, 3	
MCT1*	YOR221C	Malonyl-CoA:ACP Transferase: Predicted malonyl-CoA:ACP transferase, putative component of a type-II mitochondrial fatty acid synthase that produces intermediates for phospholipid remodeling	2	
MDJ1*	YFL016C	Protein involved in folding of mitochondrially synthesized proteins in the mitochondrial matrix; localizes to the mitochondrial inner membrane; member of the DnaJ family of molecular chaperones; MGD after 20 generations, SGD after 60 Generations, MGD on NaCl	3	
MDM10*	YAL010C	Mitochondrial Distribution and Morphology: Subunit of the mitochondrial sorting and assembly machinery (SAM complex); has a role in assembly of the TOM complex, which mediates protein import through the outer membrane; required for normal mitochondrial morphology and inheritance	3, 3	
MDM12	YOL009C	Mitochondrial Distribution and Morphology: Required for normal mitochondrial morphology and distribution; Mdm12p is a mitochondrial outer membrane protein. An Mdm12p homolog exists in S. Pombe which confers a dominant negative phenotype when expressed in S. cerevisiae	3, 3	SSS*
MDM20*	YOL076W	Subunit of the NatB N-terminal acetyltransferase, which catalyzes acetylation of the amino-terminal methionine residues of all proteins beginning with Met-Asp or Met-Glu and of some proteins beginning with Met-Asn or Met-Met; involved in mitochondrial inheritance and actin assembly	3	
MDM30	YLR368W	Protein involved in determination of mitochondrial structure	1	
MDM34	YGL219C	Mitochondrial Distribution and Morphology: Mitochondrial outer membrane protein, colocalizes with mtDNA nucleids, required for mitochondria shape; MGD after 20 generations, MGD on NaCl	1	
MDM39	YGL020C	Mitochondrial Distribution and Morphology: Protein involved in determination of mitochondrial structure	2	SSS
MEF1	YLR069C	Mitochondrial Elongation Factor: mitochondrial elongation factor G-like protein	2, 2	
MEF2	YJL102W	Mitochondrial Elongation Factor: mitochondrial elongation factor G-like protein	2, 2	
MEH1	YKR008C	Multicopy suppressor of Ers1 Hygromycin B sensitivity	2	
MET7	YOR241W	METhionine requiring: Folylpolyglutamate synthetase, catalyzes extension of the glutamate chains of the folate coenzymes, required for methionine synthesis and for maintenance of mitochondrial DNA, present in both the cytoplasm and mitochondria	3	SSS*
MET22	YOL064C	METhionine requiring: Bisphosphate-3'-nucleotidase, involved in salt tolerance and methionine biogenesis; dephosphorylates 3'-phosphoadenosine-5'-phosphate and 3'-phosphoadenosine-5'-phosphosulfate, intermediates of the sulfate assimilation pathway	2	
MGM1	YOR211C	Mitochondrial GTPase related to dynamin, present in a complex containing Ugo1p and Fzo1p; required for normal morphology of cristae and for stability of Tim11p; homolog of human OPA1 involved in autosomal dominant optic atrophy	3, 2	SSS*
MGM101	YJR144W	Mitochondrial Genome Maintenance: Involved in mitochondrial genome maintenance; (putative) nucleic acid interactor	1, 2	
MHR1	YDR296W	Mitochondrial Homologous DNA Recombination: Protein involved in mitochondrial homologous DNA recombination and in transcription regulation; binds to activation domains of acidic activators; presence in RNA pol II holoenzyme may help recruit an Ssn3p-active form of the holoenzyme to target promoters	2, 2	S, SS*
MID2	YLR332W	Protein required for mating	1	

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MIG1	YGL035C	Multicopy Inhibitor of GAL gene expression: Transcription factor involved in glucose repression; C2H2 zinc finger protein similar to mammalian Egr and Wilms tumor proteins; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2	SS
MIP1	YOR330C	catalytic subunit of mitochondrial DNA polymerase	1, 2	SS*
MKS1	YNL076W	negative transcriptional regulator; Pleiotropic regulatory factor involved in Ras-CAMP and lysine biosynthetic pathways and nitrogen regulation; involved in retrograde (RTG) mitochondria-to-nucleus signaling, SGD after20 & 60 generations	2	
MMM1	YLL006W	mitochondrial outer membrane protein: Involved in mechanism by which mitochondrial shape is established or maintained	2, 2	
MMS22*	YLR420W	Methyl MethaneSulfonate sensitivity	3	
MNN2	YBR015C	MaNNosyltransferase: Alpha-1,2-mannosyltransferase, responsible for addition of the first alpha-1,2-linked mannose to form the branches on the mannan backbone of oligosaccharides, localizes to an early Golgi compartment; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2	
MNN9	YPL050C	Subunit of Golgi mannosyltransferase complex also containing Anp1p, Mnn10p, Mnn11p, and Hoc1p that mediates elongation of the polysaccharide mannan backbone; forms a separate complex with Van1p that is also involved in backbone elongation	3, 3	SSS
MNN10	YDR245W	Subunit of a Golgi mannosyltransferase complex also containing Anp1p, Mnn9p, Mnn11p, and Hoc1p that mediates elongation of the polysaccharide mannan backbone; membrane protein of the mannosyltransferase family	2	
MNN11*	YJL183W	MaNNosyltransferase: Subunit of a Golgi mannosyltransferase complex that also contains Anp1p, Mnn9p, Mnn10p, and Hoc1p, and mediates elongation of the polysaccharide mannan backbone; has homology to Mnn10p	2	
MON2	YNL297C	MONensin sensitivity: Peripheral membrane protein with a role in endocytosis and vacuole integrity, interacts with Arl1p and localizes to the endosome; member of the Sec7p family of proteins	3	
MRC1	YCL061C	S-phase checkpoint protein found at replication forks, required for DNA replication; also required for Rad53p activation during DNA replication stress, where it forms a replication-pausing complex with Tof1p and is phosphorylated by Mec1p; protein involved in replication checkpoint	1	SSS, SS*
MRF1	YGL143C	Mitochondrial peptide chain Release Factor: Mitochondrial polypeptide chain release factor, involved in stop codon recognition and hydrolysis of the peptidyl-tRNA bond during mitochondrial translation; lack of MRF1 causes mitochondrial genome instability; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1, 2	
MRH4	YGL064C	Mitochondrial RNA Helicase: mitochondrial DEAD box RNA helicase, plays an essential role in mitochondrial function; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1, 3	S
MRM1	YOR201C	Ribose methyltransferase that modifies a functionally critical, conserved nucleotide in mitochondrial 21S rRNA	2, 2	
MRP1	YDR347W	Mitochondrial Ribosomal Protein: Mitochondrial ribosomal protein of the small subunit; MRP1 exhibits genetic interactions with PET122, encoding a COX3-specific translational activator, and with PET123, encoding a small subunit mitochondrial ribosomal protein	2, 3	
MRP2	YPR166C	Mitochondrial ribosomal protein of the small subunit	2	SS*
MRP7	YNL005C	Mitochondrial Ribosomal Protein: Mitochondrial ribosomal protein of the large subunit Also known as: MRPL2, YmL2; SGD after 20 & 60 generations, MGD on NaCl	3	
MRP10*	YDL045W-A	Mitochondrial ribosomal protein of the small subunit	2, 3	SSS
MRP17	YKL003C	Mitochondrial ribosomal protein of the small subunit; MRP17 exhibits genetic interactions with PET122, encoding a COX3-specific translational activator; SGD after 20 & 60 generations, SGD on NaCl	1, 2	
MRP20	YDR405W	Mitochondrial ribosomal protein of the large subunit	2, 3	S
MRP21	YBL090W	Mitochondrial ribosomal protein of the large subunit; MRP21 exhibits genetic interactions with mutations in the COX2 and COX3 mRNA 5'-untranslated leader sequences	1, 2	
MRP49	YKL167C	Mitochondrial ribosomal protein of the large subunit, not essential for mitochondrial translation	2, 2	
MRP51	YPL118W	Mitochondrial ribosomal protein of the large subunit; MRP51 exhibits genetic interactions with mutations in the COX2 and COX3 mRNA 5'-untranslated leader sequences	2, 2	SS*
MRPL6	YHR147C	Mitochondrial ribosomal protein of the large subunit Also known as: YmL16; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1, 2	
MRPL7	YDR237W	Mitochondrial ribosomal protein of the large subunit Also known as: YmL5/7	1, 2	S, SS*
MRPL8	YJL063C	Mitochondrial ribosomal protein of the large subunit Also known as: YmL8	2, 2	
MRPL9	YGR220C	Mitochondrial ribosomal protein of the large subunit Also known as: YmL9	1, 2	
MRPL10	YNL284C	Mitochondrial ribosomal protein of the large subunit; appears as two protein spots (YmL10 and YmL18) on two-dimensional SDS gels Also known as: MRPL18, YmL10, YmL18	2, 3	
MRPL11	YDL202W	Mitochondrial ribosomal protein of the large subunit	2, 2	
MRPL13	YKR006C	Mitochondrial ribosomal protein of the large subunit, not essential for mitochondrial translation Also known as: YmL13	1, 2	
MRPL15	YLR312W-A	Mitochondrial ribosomal protein of the large subunit Also known as: YmL15	1, 2	SS*
MRPL16	YBL038W	Mitochondrial ribosomal protein of the large subunit	2, 2	
MRPL17	YNL252C	Mitochondrial ribosomal protein of the large subunit Also known as: MRPL30, YmL30	2, 2	
MRPL20	YKR085C	Mitochondrial Ribosomal Protein, Large subunit: SGD after 20 & 60 generations, SGD on NaCl	2, 3	
MRPL23	YOR150W	Mitochondrial ribosomal protein of the large subunit Also known as: YmL23; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1, 3	SS
MRPL24	YMR193W	Mitochondrial ribosomal protein of the large subunit Also known as: MRPL14, YmL14, YmL24	1, 2	S*
MRPL25	YGR076C	Mitochondrial ribosomal protein of the large subunit Also known as: YmL25	2, 2	
MRPL27	YBR282W	Mitochondrial ribosomal protein of the large subunit	2, 3	SSS
MRPL28	YDR462W	Mitochondrial ribosomal protein of the large subunit. Also known as: YmL28	2, 3	S
MRPL32	YCR003W	Mitochondrial ribosomal protein of the large subunit	2, 3	
MRPL33	YMR286W	Mitochondrial ribosomal protein of the large subunit	1, 2	SS*
MRPL35	YDR322W	Mitochondrial ribosomal protein of the large subunit	2, 2	
MRPL37	YBR268W	Mitochondrial ribosomal protein of the large subunit. Also known as: YmL37	2, 2	

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MRPL38	YKL170W	Mitochondrial ribosomal protein of the large subunit; appears as two protein spots (YmL34 and YmL38) on two-dimensional SDS gels. Also known as: MRPL34, YmL38, YmL34; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2, 2	
MRPL40	YPL173W	Mitochondrial ribosomal protein of the large subunit	1, 3	S*
MRPL49	YJL096W	Mitochondrial ribosomal protein of the large subunit Also known as: YmL49; MGD after 20 & 60 generations, MGD on NaCl	2, 3	
MRPL51	YPR100W	Mitochondrial ribosomal protein of the large subunit	1, 2	S*
MRPS5	YBR251W	Mitochondrial ribosomal protein of the small subunit	2, 2	
MRPS16	YPL013C	Mitochondrial ribosomal protein of the small subunit	1, 2	S*
MRPS17	YMR188C	Mitochondrial ribosomal protein of the small subunit	1, 2	SS*
MRPS28	YDR337W	Mitochondrial ribosomal protein of the small subunit	1, 3	
MRPS35*	YGR165W	Mitochondrial ribosomal protein of the small subunit	1, 2	S, SSS*
MRS1	YIR021W	Mitochondrial RNA Splicing: Protein required for the splicing of two mitochondrial group I introns (BI3 in COB and AI5beta in COX1); forms a splicing complex, containing four subunits of Mrs1p and two subunits of the BI3-encoded maturase, that binds to the BI3 RNA; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1, 2	
MSC2	YDR205W	Meiotic Sister-Chromatid recombination: Member of the cation diffusion facilitator family, localizes to the endoplasmic reticulum and nucleus; mutations affect the cellular distribution of zinc and also confer defects in meiotic recombination between homologous chromatids; MGD after 20 & 60 generations	1	
MSD1	YPL104W	Mitochondrial aspartyl-tRNA synthetase, required for acylation of aspartyl-tRNA; yeast and bacterial aspartyl-, asparaginyl-, and lysyl-tRNA synthetases contain regions with high sequence similarity, suggesting a common ancestral gene	3, 2	S, SSS*
MSE1	YOL033W	Mitochondrial glutamyl-tRNA synthetase, encoded by a nuclear gene	2, 2	SS*
MSF1	YPR047W	Mitochondrial phenylalanyl-tRNA synthetase alpha subunit, active as a monomer, unlike the cytoplasmic subunit which is active as a dimer complexed to a beta subunit dimer; similar to the alpha subunit of E. coli phenylalanyl-tRNA synthetase	1, 2	
MSG5	YNL053W	Multicopy Suppressor of GPA1: Dual-specificity protein phosphatase required for maintenance of a low level of signaling through the cell integrity pathway; regulates and is regulated by Slt2p; also required for adaptive response to pheromone; SGD after 20 & 60 generations, MGD on NaCl	1	
MSH1	YHR120W	DNA-binding protein of the mitochondria involved in repair of mitochondrial DNA, has ATPase activity and binds to DNA mismatches; has homology to E. coli MutS; transcription is induced during meiosis	3, 2	
MSK1	YNL073W	Mitochondrial aminoacyl-tRNA Synthetase, lysine (K): Mitochondrial lysine-tRNA synthetase, required for import of both aminoacylated and deacylated forms of tRNA(Lys) into mitochondria; SGD after 20 & 60 generations, SGD on NaCl	3	
MSM1	YGR171C	Mitochondrial aminoacyl-tRNA Synthetase, Methionine: Mitochondrial methionyl-tRNA synthetase (MetRS), functions as a monomer in mitochondrial protein synthesis; functions similarly to cytoplasmic MetRS although the cytoplasmic form contains a zinc-binding domain not found in Msm1p	2	S, SS*
MSO1	YNR049C	Multicopy suppressor of Sec One: Probable component of the secretory vesicle docking complex, acts at a late step in secretion; shows genetic and physical interactions with Sec1p and is enriched in microsomal membrane fractions; required for sporulation; MGD at 20 & 60 generations, MGD on NaCl	1	
MSR1	YHR091C	Nuclear-encoded mitochondrial protein; Arginyl-tRNA synthetase	1, 2	
MSS2	YDL107W	Mitochondrial Splicing System: Peripherally bound inner membrane protein of the mitochondrial matrix, required for export of C-terminal tail of Cox2p through the inner membrane	1, 2	
MSS51	YLR203C	Mitochondrial Splicing System: Protein required for the maturation and translation of COX1 mRNA; involved in maturation of COX1 and COB mRNA	2, 2	
MSS116	YDR194C	Mitochondrial Splicing System: DEAD-box protein required for efficient splicing of mitochondrial Group I and II introns; presumed RNA helicase due to DEAD-box motif	3	SSS
MSU1	YMR287C	RNase, component of the mitochondrial degradosome along with the ATP-dependent RNA helicase Suv3p; the degradosome associates with the ribosome and mediates turnover of aberrant or unprocessed RNAs	1, 2	SS*
MSW1	YDR268W	Mitochondrial tryptophanyl-tRNA synthetase	2	S*
MSY1	YPL097W	Tyrosyl-tRNA synthetase	1, 3	SS*
MTF1	YMR228W	Mitochondrial Transcription Factor: Mitochondrial RNA polymerase sigma-like specificity factor required for promoter recognition, interacts with mitochondrial core polymerase Rpo41p, imported into mitochondria via a novel process requiring most of the Mtf1p sequence	1, 2	SS*
MTF2	YDL044C	Mitochondrial Transcription Factor: Mitochondrial matrix protein that interacts with an N-terminal region of mitochondrial RNA polymerase (Rpo41p) and couples RNA processing and translation to transcription	2, 3	SSS
MTG1	YMR097C	Peripheral GTPase of the mitochondrial inner membrane, essential for respiratory competence, likely functions in assembly of the large ribosomal subunit, has homologs in plants and animals	2, 2	
MTG2	YHR168W	MiTochondrial Gtpase 2: Putative GTPase, member of the Obg family; peripheral protein of the mitochondrial inner membrane that associates with the large ribosomal subunit; required for mitochondrial translation, possibly via a role in ribosome assembly	1, 2	
MTM1	YGR257C	Manganese Trafficking factor for Mitochondrial SOD2: Mitochondrial protein of the mitochondrial carrier family, involved in activating mitochondrial Sod2p probably by facilitating insertion of an essential manganese cofactor; SGD after 20 & 60 generations, MGD on NaCl	2, 3	SSS
MTO1	YGL236C	Mitochondrial protein required for respiration in paromomycin-resistant 15S rRNA mutants; forms a heterodimer complex with Mss1p that plays a role in optimizing mitochondrial protein synthesis	2	SSS
MUD2	YKL074C	Protein involved in early pre-mRNA splicing; component of the pre-mRNA-U1 snRNP complex, the commitment complex; interacts with Msl5p/BBP splicing factor and Sub2p; similar to metazoan splicing factor U2AF65	2	
NAB6	YML117W	Putative RNA-binding protein, based on computational analysis of large-scale protein-protein interaction data	1	SSS, SSS*
NBP2	YDR162C	Nap1 Binding Protein: Protein involved in the HOG (high osmolarity glycerol) pathway, negatively regulates Hog1p by recruitment of phosphatase Ptc1p the Pbs2p-Hog1p complex, found in the nucleus and cytoplasm, contains an SH3 domain that binds Pbs2p	2	
NCE4	YPL024W	Negative regulator of Cts1 Expression: Protein of unknown function; GFP tagged protein localizes to the cytoplasm and nucleus	1	SSS, SS*
NCL1	YBL024W	S-adenosyl-L-methionine-dependent tRNA: m5C-methyltransferase, methylates cytosine to m5C at several positions in tRNAs and intron-containing pre-tRNAs; similar to Nop2p and human proliferation associated nucleolar protein p120	2	
NEM1	YHR004C	Nuclear Envelope Morphology	3	
NGG1	YDR176W	Involved in glucose repression of GAL4p-regulated transcription; transcription factor; genetic and mutant analyses suggest that Ngg1p (Ada3p) is part of two transcriptional adaptor/HAT (histone acetyltransferase complexes, the 0.8 MD ADA complex and the 1.8 MD SAGA complex	2	
NGR1	YBR212W	negative growth regulatory protein	1	S, S*
NIP100	YPL174C	Nuclear import protein; (putative) large subunit of dynactin complex	1	SSS, SS*

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NKP2	YLR315W	Non-essential Kinetochore Protein	1	
NOT4* (MOT2)	YER068W	Component of the CCR4-NOT transcription regulatory complex, which represses transcription, at least in part, by inhibiting functional TBP-DNA interactions and also aids in transcription elongation; interacts with C-terminal region of Not1p; MGD after 20 generations, SGD after 60 generations, SGD on NaCl	2, 3	SSS
NOT5*	YPR072W	Subunit of the CCR4-NOT complex, which is a global transcriptional regulator with roles in transcription initiation and elongation and in mRNA degradation	3, 3	S, SSS*
NPL4	YBR170C	Nuclear Protein Localization: Nuclear protein that may have a role in nuclear protein import, MGD after 20 & 60 gewnerations, MGD on NaCl	1	
NPL6	YMR091	Nuclear Protein Localization: Nuclear protein that may have a role in nuclear protein import, MGD after 20 & 60 gewnerations	3	
NPT1	YOR209C	Nicotinate PhosphoribosylTransferase: Nicotinate phosphoribosyltransferase, acts in the salvage pathway of NAD+ biosynthesis; required for silencing at rDNA and telomeres and has a role in silencing at mating-type loci; localized to the nucleus	1	SSS, SSS*
NUM1	YDR150W	NUclear Migration: Protein required for nuclear migration, localizes to the mother cell cortex and the bud tip; may mediate interactions of dynein and cytoplasmic microtubules with the cell cortex	1	S*
OAR1	YKL055C	3-Oxoacyl-[Acyl-carrier-protein] Reductase: Mitochondrial 3-oxoacyl-[acyl-carrier-protein] reductase, may comprise a type II mitochondrial fatty acid synthase along with Mct1p	3	
OCA1	YNL099C	Oxidant-induced Cell-cycle Arrest: Putative protein tyrosine phosphatase, required for cell cycle arrest in response to oxidative damage of DNA; SGD after 20 & 60 generations, MGD on NaCl	2	
OCH1*	YGL038C	Outer CHain elongation: Mannosyltransferase of the cis-Golgi apparatus, initiates the polymannose outer chain elongation of N-linked oligosaccharides of glycoproteins	3, 3	SSS
0CT1	YKL134C	OCTapeptidyl aminopeptidase: Mitochondrial intermediate peptidase, cleaves N-terminal residues of a subset of proteins upon import, after their cleavage by mitochondrial processing peptidase (Mas1p-Mas2p); may contribute to mitochondrial iron homeostasis	3, 2	
OPI3	YJR073C	Phospholipid methyltransferase (methylene-fatty-acyl-phospholipid synthase), catalyzes the last two steps in phosphatidylcholine biosynthesis	3	
OPT1	YJL212C	OligoPeptide Transporter: Plasma membrane transporter that transports tetra- and pentapeptides and glutathione; member of the OPT family	2	
OST3	YOR085W	OligoSaccharylTransferase: Gamma subunit of the oligosaccharyltransferase complex of the ER lumen, which catalyzes aspargine-linked glycosylation of newly synthesized proteins; Ost3p is important for N-glycosylation of a subset of proteins	2	
OST6	YML019W	OligoSaccharylTransferase: Subunit of the oligosaccharyltransferase complex of the ER lumen, which catalyzes aspargine-linked glycosylation of newly synthesized proteins; similar to and partially functionally redundant with Ost3p	2	
OXA1	YER154W	cytochrome OXidase Activity: Translocase of the mitochondrial inner membrane, mediates the insertion of both mitochondrial- and nuclear-encoded proteins from the matrix into the inner membrane, interacts with mitochondrial ribosomes; null is respiratory deficient; Also known as: PET1402	3	SSS
PAC11	YDR488C	Perishes in the Absence of CIN8: Dynein intermediate chain, acts in the cytoplasmic dynein pathway, forms cortical cytoplasmic microtubule capture site with Num1p; null mutant is defective in nuclear migration, essential in the absence of CIN8	2	S; SSS*
PAF1*	YBR279W	RNA Polymerase-Associated Factor: RNA polymerase II-associated protein, defines a large complex that is biochemically and functionally distinct from the Srb-Mediator form of Pol II holoenzyme and is required for full expression of a subset of cell cycle-regulated genes	2, 3	SSS
PCP1	YGR101W	Mitochondrial serine protease required for the processing of various mitochondrial proteins and maintenance of mitochondrial DNA and morphology; belongs to the rhomboid-GlpG superfamily of intramembrane peptidases	3, 2	
PDB1	YBR221C	E1 beta subunit of the pyruvate dehydrogenase (PDH) complex, which is an evolutionarily-conserved multi-protein complex found in mitochondria	1	SS, SS*
PDE2	YOR360C	High-affinity cyclic AMP phosphodiesterase, component of the cAMP-dependent protein kinase signaling system, protects the cell from extracellular cAMP, contains readthrough motif surrounding termination codon	2	
PEP3	YLR148W	carboxyPEPtidase Y-deficient: Vacuolar peripheral membrane protein that promotes vesicular docking/fusion reactions in conjunction with SNARE proteins, required for vacuolar biogenesis, forms complex with Pep5p that mediates protein transport to the vacuole; MGD after 20 generations SGD after 60 Generations, MGD on NaCl	3, 3	
PEP7	YDR325C	Multivalent adaptor protein that facilitates vesicle-mediated vacuolar protein sorting by ensuring high-fidelity vesicle docking and fusion, which are essential for targeting of vesicles to the endosome; required for vacuole inheritance	2	
PEP8	YJL053W	carboxyPEPtidase Y-deficient: Vacuolar protein sorting protein that forms part of the multimeric membrane-associated retromer complex along with Vps35p, Vps29p, Vps17p, and Vps5p; essential for endosome-to-Golgi retrograde protein transport	3	
PEP12	YOR036W	carboxyPEPtidase Y-deficient: Target membrane receptor (t-SNARE) for vesicular intermediates traveling between the Golgi apparatus and the vacuole; controls entry of biosynthetic, endocytic, and retrograde traffic into the prevacuolar compartment; syntaxin	2	
PER1	YCR044C	Vacuolar membrane protein that seems to be involved in Mn2+ homeostasis; mutant is dependent on activation of the unfolded protein response pathway for viability	2, 3	SSS, SSS*
PET54	YGR222W	Protein required for splicing of the COX1 intron AI5 beta; also specifically required, together with Pet122p and Pet494p, for translation of the COX3 mRNA; located in the mitochondrial inner membrane	3, 2	
PET100	YDR079W	Chaperone that specifically facilitates the assembly of cytochrome c oxidase, located in the mitochondrial inner membrane	1, 2	S, SS*
PET112	YBL080C	Protein required for mitochondrial translation; mutation is functionally complemented by a Bacillus subtilis ortholog	1, 2	
PET130	YJL023C	Protein required for respiratory growth	2	
PET191	YJR034W	PETite colonies: Protein required for assembly of cytochrome c oxidase	3, 3	
PET309	YLR067C	PETite colonies: Specific translational activator for the COX1 mRNA, also influences stability of intron-containing COX1 primary transcripts; located in the mitochondrial inner membrane; MGD after 20 & 60 generations, MGD on NaCl	2, 2	
PEX5	YDR244W	PEroXisome related: Essential for import of proteins with SKL-like import signal into peroxisomal matrix; 69-kDa protein containing tetratricopeptide repeat (TPR)	2	
PEX11	YOL147C	PEroXisome related: Peroxisomal membrane protein required for peroxisome proliferation and medium-chain fatty acid oxidation, most abundant protein in the peroxisomal membrane, regulated by Adr1p and Pip2p-Oaf1p, promoter contains ORE and UAS1-like elements	2	
PEX14	YGL153W	PEroXisome related: Peroxisomal peripheral membrane protein (peroxin) involved in import of peroxisomal matrix proteins; component of peroxisomal import machinery; MGD after 20 & 60 generations, MGD on NaCl	2	
PFK2*	YMR205C	PhosphoFructoKinase: Beta subunit of heterooctameric phosphofructokinase involved in glycolysis, indispensable for anaerobic growth, activated by fructose-2,6-bisphosphate and AMP, mutation inhibits glucose induction of cell cycle-related genes	1	SSS, SSS*

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PFK26	YIL107C	6-PhosphoFructo-2-Kinase: 6-phosphofructo-2-kinase, inhibited by phosphoenolpyruvate and sn-glycerol 3-phosphate, has negligible fructose-2,6-bisphosphatase activity, transcriptional regulation involves protein kinase A; Also known as: PFK-2; MGD after 20 & 60 generations, MGD on NaCl	2	
PGD1	YGL025C	PolyGlutamine Domain: Subunit of the Mediator global transcriptional cofactor complex, which is part of the RNA polymerase II holoenzyme and plays an essential role in basal and activated transcription; direct target of the Cyc8p-Tup1p transcriptional corepressor	2	S
PGM1	YKL127W	phosphoglucomutase, minor isoform	2	
PHO2	YDL106C	PHOsphate metabolism: Homeobox transcription factor; regulatory targets include genes involved in phosphate metabolism; binds cooperatively with Pho4p to the PHO5 promoter; phosphorylation of Pho2p facilitates interaction with Pho4p	2, 3	
PHO80	YOL001W	PHOsphate metabolism: Cyclin, negatively regulates phosphate metabolism; Pho80p-Pho85p (cyclin-CDK complex) phosphorylates Pho4p and Swi5p; deletion of PHO80 leds to aminoglycoside supersensitivity; truncated form of PHO80 affects vacuole inheritance	2	
PHO84	YML123C	PHOsphate metabolism: High-affinity inorganic phosphate (Pi) transporter and low-affinity manganese transporter; regulated by Pho4p and Spf7p; mutation confers resistance to arsenate; exit from the ER during maturation requires Pho86p	2	
PHO85	YPL031C	PHOsphate metabolism: Cyclin-dependent kinase, with ten cyclin partners; involved in environmental stress response; in phosphate-rich conditions, Pho85p-Pho80p complex phosphorylates Pho4p which in turn represses PHO5	2	
PHO88	YBR106W	PHOsphate metabolism: Probable membrane protein, involved in phosphate transport; pho88 pho86 double null mutant exhibits enhanced synthesis of repressible acid phosphatase at high inorganic phosphate concentrations; MGD after20 & 60 generations, MGD on NaCl	1	
PIF1	YML061C	5'-3' DNA helicase: DNA helicase involved in telomere formation and elongation; acts as a catalytic inhibitor of telomerase; also plays a role in repair and recombination of mitochondrial DNA	2, 2	SS*
PIL1	YGR092W	Phosphorylation Inhibited by Long chain bases: Long chain base-responsive inhibitor of protein kinases Phk1p and Phk2p, acts along with Lsp1p to down-regulate heat stress resistance via regulation of the Pkc1p and Ypk1p pathways; phosphorylated by Phk1p and Phk2p; SGD after 20 & 60 generations, SGD on NaCl	1	
PIN2	YOR104W	Psi+ Inducibility: [PSI+] induction	1	
PIN4	YBL051C	Protein involved in G2/M phase progression and response to DNA damage, interacts with Rad53p; contains an RNA recognition motif, a nuclear localization signal, and several SQ/TQ cluster domains; hyperphosphorylated in response to DNA damage	1	SSS, SS*
PKR1	YMR123W	Pichia farinosa Killer toxin Resistance; Pichia farinosa killer toxin resistance	2	
PMP3	YDR276C	Plasma Membrane Proteolipid: plasma membrane protein involved in salt tolerance; hypothetical transmembrane protein	2	
PMR1	YGL167C	Plasma Membrane ATPase Related: High affinity Ca2+/Mn2+ P-type ATPase, required for Ca2+ and Mn2+ transport into Golgi; involved in Ca2+ dependent protein sorting and processing; mutations in human homolog ATP2C1 cause acantholytic skin condition Hailey-Hailey disease; SGD after 20 & 60 generations	3, 3	
PMT2	YAL023C	Transfers mannosyl residues from dolichyl phosphate-D-mannose to seryl and threonyl residues in proteins; acts in complex with Pmt1p; dolichyl phosphate-D-mannose:protein O-D-mannosyltransferase	3	
POG1	YIL122W	Promoter of Growth: weak similarity to human transcription adaptor protein p300	2	
POP2*	YNR052C	PGK promoter directed OverProduction: RNase of the DEDD superfamily, subunit of the Ccr4-Not complex that mediates 3' to 5' mRNA deadenylation	3	
POR1	YNL055C	Mitochondrial porin (voltage-dependent anion channel), outer membrane protein required for the maintenance of mitochondrial osmotic stability and mitochondrial membrane permeability, Also known as: YVDAC1; SGD after 20 & 60 generations, MGD on NaCl	2	
PPA2	YMR267W	Mitochondrial inorganic pyrophosphatase, required for mitochondrial function and possibly involved in energy generation from inorganic pyrophosphate	1, 2	S*
PPG1	YNR032W	Putative serine/threonine protein phosphatase, required for glycogen accumulation; interacts with Tap42p, which binds to and regulates other protein phosphatases	1	
PPM1	YDR435C	Protein Phosphatase Methyltransferase: Carboxyl methyl transferase, methylates the C terminus of the protein phosphatase 2A catalytic subunit (Pph21p or Pph22p), which is important for complex formation with regulatory subunits	2	
PRO2	YOR323C	PROline requiring: Gamma-glutamyl phosphate reductase, catalyzes the second step in proline biosynthesis	3	
PRP18	YGR006W	Pre-mRNA Processing: Splicing factor involved in the positioning of the 3' splice site during the second catalytic step of splicing, recruited to the spliceosome by Slu7p	2, 3	SS, SS*
PRS5	YOL061W	5-phospho-ribosyl-1(alpha)-pyrophosphate synthetase, involved in nucleotide, histidine, and tryptophan biosynthesis; one of a five related enzymes, which are active as heteromultimeric complexes	1	
PRR1	YKL116C	Pheromone Response Regulator: Protein kinase with a possible role in MAP kinase signaling in the pheromone response pathway	3	
PTC1	YDL006W	Phosphatase type Two C: Type 2C protein phosphatase (PP2C); inactivates the osmosensing MAPK cascade by dephosphorylating Hog1p; mutation delays mitochondrial inheritance; deletion reveals defects in precursor tRNA splicing, sporulation and cell separation	3	
QCR2	YPR191W	ubiQuinol-cytochrome C oxidoReductase: 40 kDa ubiquinol cytochrome-c reductase core protein 2	2, 2	SS*
QCR7	YDR529C	ubiQuinol-cytochrome C oxidoReductase: ubiquinol-cytochrome c oxidoreductase subunit 7 (14 kDa)	2, 3	SSS
QCR8	YJL166W	ubiQuinol-cytochrome C oxidoReductase: Ubiquinol cytochrome-c reductase subunit 8 (11 kDa protein); MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2, 2	
QCR10	YHR001W-A	ubiQuinol-cytochrome C oxidoReductase: 8.5 kDa subunit of the ubiquinol-cytochrome c oxidoreductase complex	3	
RAD6*	YGL058W	RADiation sensitive: Ubiquitin-conjugating enzyme (E2), involved in postreplication repair (with Rad18p), sporulation, telomere silencing, and ubiquitin-mediated N-end rule protein degradation (with Ubr1p); MGD after 20 & 60 generations, SGD on NaCl	3	SS*
RAD27*	YKL113C	RADiation sensitive: 5' to 3' exonuclease, 5' flap endonuclease, required for Okazaki fragment processing and maturation as well as for long-patch base-excision repair; member of the S. pombe RAD2/FEN1 family	2	
RAD50*	YNL250W	RADiation sensitive: Subunit of MRX complex, with Mre11p and Xrs2p, involved in processing double-strand DNA breaks in vegetative cells, initiation of meiotic DSBs, telomere maintenance, and nonhomologous end joining	2	
RAD51*	YER095W	RADiation sensitive: Strand exchange protein, forms a helical filament with DNA that searches for homology; involved in the recombinational repair of double-strand breaks in DNA during vegetative growth and meiosis; homolog of Dmc1p and bacterial RecA protein	2	
RAD52*	YML032C	RADiation sensitive: Protein that stimulates strand exchange by facilitating Rad51p binding to single-stranded DNA; anneals complementary single-stranded DNA; involved in the repair of double-strand breaks in DNA during vegetative growth and meiosis	1	
RAD54*	YGL163C	RADiation sensitive: DNA-dependent ATPase, stimulates strand exchange by modifying the topology of double-stranded DNA; involved in the recombinational repair of double-strand breaks in DNA during vegetative growth and meiosis; member of the SWI/SNF family; MGD after 20 generations; SGD after 60 generations, MGD on NaCl	2	
RAD61*	YDR014W	RADiation sensitive: Protein of unknown function; mutation confers radiation sensitivity	1	

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RAV1	YJR033C	Regulator of (H+)-ATPase in Vacuolar membrane: Subunit of the RAVE complex (Rav1p, Rav2p, Skp1p), which promotes assembly of the V-ATPase holoenzyme; required for transport between the early and late endosome/PVC and for localization of TGN membrane proteins; potential Cdc28p substrate	1	
RAV2	YDR202C	Regulator of (H+)-ATPase in Vacuolar membrane: Subunit of RAVE (Rav1p, Rav2p, Skp1p), a complex that associates with the V1 domain of the vacuolar membrane (H+)-ATPase (V-ATPase) and promotes assembly and reassembly of the holoenzyme	1	
RCE1	YMR274C	Protease involved in ras and a-factor terminal proteolysis; protease, acts on Ras and a-factor C-termini	2	
RCY1	YJL204C	ReCYcling 1: Deletion leads to an early block in the endocytic pathway before the intersection with the vacuolar protein sorting pathway	3	
REF2*	YDR195W	RNA End Formation: RNA-binding protein involved in the cleavage step of mRNA 3'-end formation prior to polyadenylation; also involved in snoRNA maturation	1, 3	SSS
REG1	YDR028C	REsistance to Glucose repression: Regulatory subunit of type 1 protein phosphatase Glc7p, involved in negative regulation of glucose-repressible genes; involved in RNA processing	1	S, S*
RER1	YCL001W	Retention in the Endoplasmic Reticulum: Protein involved in retention of membrane proteins, including Sec12p, in the ER; localized to Golgi; functions as a retrieval receptor in returning membrane proteins to the ER	1	S, SS*
RFM1	YOR279C	DNA-binding protein required for repression of middle sporulation genes; specificity factor that directs the Hst1p histone deacetylase to some of the promoters regulated by Sum1p	2	
RGP1	YDR137W	Reduced Growth Phenotype: Subunit of a Golgi membrane exchange factor (Ric1p-Rgp1p) that catalyzes nucleotide exchange on Ypt6p	3	
RHK1	YBL082C	Dolichol-P-Man dependent alpha(1-3) mannosyltransferase, involved in the synthesis of dolichol-linked oligosaccharide donor for N-linked glycosylation of proteins	2	
RHO2	YNL090W	Ras Homolog: Non-essential small GTPase of the Rho/Rac subfamily of Ras-like proteins, involved in the establishment of cell polarity and in microtubule assembly, MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1	
RIB1	YBL033C	RIBoflavin biosynthesis: GTP cyclohydrolase II; catalyzes the first step of the riboflavin biosynthesis pathway; MGD after 20 generations, SGD after 60 generations, SGD on NaCl	3	
RIC1	YLR039C	RIbosome Control: Subunit of a Golgi membrane exchange factor (Ric1p-Rgp1p) that catalyzes nucleotide exchange on Ypt6p; involved in transcription of ribosomal protein genes and ribosomal RNA	2	
RIM1*	YCR028C-A	Replication In Mitochondria: Single-stranded DNA-binding protein that is essential for mitochondrial genome maintenance; Single-stranded zinc finger DNA-binding protein	2	
RIM8	YGL045W	Involved in proteolytic processing of Rim1p	2	SSS
RIM9	YMR063W	Regulator of IME2: Protein required for IME1 expression; involved in sporulation	3	
RIM13	YMR154C	Regulator of IME2 (RIM); Cysteine protease similar to E. nidulans palB	3	
RIM20	YOR275C	Regulator of IME2: Protein involved in proteolytic activation of Rim101p in response to alkaline pH; member of the PalA/AIP1/Alix family; interacts with the ESCRT-III subunits Snf7p, suggesting a relationship between the response to pH and multivesicular body formation; MGS to NaCl	1	
RIM21	YNL294C	Regulator of IME2	3	
RIM101	YHL027W	Transcriptional activator required for entry into meiosis, has similarity to the Aspergillus Phenotype-response regulator PacC and the Yarrowia proteinase YlRim1010p; Meiotic regulatory protein; Cys-His zinc fingers	3	
RIP1	YEL024W	Rieske Iron-sulfur Protein: oxidizes ubiquinol at center P in the protonmotive Q cycle mechanism, transferring one electron to cytochrome c1 and generating a low-potential ubisemiquinone anion which reduces the low-potential cytochrome b-566 heme group; Rieske iron-sulfur protein of the mitochondrial cytochrome bc1 complex	2, 2	SS*
RMD7	YER083C	Required for Meiotic nuclear Division; functions in DNA replication and damage response; Protein involved in cell wall function	2	SSS
RMD9	YGL107C	Required for Meiotic nuclear Division: Mitochondrial protein required for sporulation; MGD after 20 generations, SGD after 60 generations	1, 3	
RML2	YEL050C	Mitochondrial ribosomal protein of the large subunit, has similarity to E. coli L2 ribosomal protein; fat21 mutant allele causes inability to utilize oleate and may interfere with activity of the Adr1p transcription factor	2, 2	
RNR1	YER070W	RiboNucleotide Reductase: Ribonucleotide-diphosphate reductase (RNR), large subunit; the RNR complex catalyzes the rate-limiting step in dNTP synthesis and is regulated by DNA replication and DNA damage checkpoint pathways via localization of the small subunits; SGD after 20 & 60 generations, MGD on NaCl	3	
RNR4	YGR180C	Ribonucleotide-diphosphate reductase (RNR), small subunit; the RNR complex catalyzes the rate-limiting step in dNTP synthesis and is regulated by DNA replication and DNA damage checkpoint pathways via localization of the small subunits	2, 3	SSS, SSS*
ROM2	YLR371W	GDP/GTP exchange protein (GEP) for Rho1p and Rho2p; mutations are synthetically lethal with mutations in rom1, which also encodes a GEP	3	
ROT2	YBR229C	Reversal Of Tor2 lethality: Glucosidase II catalytic subunit required for normal cell wall synthesis; mutations in rot2 suppress tor2 mutations, and are synthetically lethal with rot1 mutations	2	
ROX3	YBL093C	Repressor Of hypoXic genes: RNA polymerase II holoenzyme component	3, 3	SSS, SSS*
RPB9*	YGL070C	SaGa associated Factor 73kDa; Probable 73KkDa Subunit of SAGA histone acetyltransferase complex	2, 3	SSS
RPL1B	YGL135W	Ribosomal Protein of the Large subunit: N-terminally acetylated protein component of the large (60S) ribosomal subunit, nearly identical to Rpl1Bp and has similarity to E. coli L1 and rat L10a ribosomal proteins; rpl1a rpl1b double null mutation is lethal; SGD after 20 & 60 generations, SGD on NaCl	1, 2	
RPL2B	YIL018W	Protein component of the large (60S) ribosomal subunit, identical to Rpl2Ap and has similarity to E. coli L2 and rat L8 ribosomal proteins; expression is upregulated at low temperatures	1	
RPL15B	YMR121C	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl15Ap and has similarity to rat L15 ribosomal protein; binds to 5.8 S rRNA		
RPL16B	YNL069C	Ribosomal Protein of the Large subunit: N-terminally acetylated protein component of the large (60S) ribosomal subunit, binds to 5.8 S rRNA; has similarity to Rpl16Ap, E. coli L13 and rat L13a ribosomal proteins; transcriptionally regulated by Rap1p; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1	
RPL22A	YLR061W	Ribosomal Protein of the Large subunit: Protein component of the large (60S) ribosomal subunit, has similarity to Rpl22Bp and to rat L22 ribosomal protein; MGD after 20 generations, SGD after 60 generations	3	
RPL31A*	YDL075W	Ribosomal Protein of the Large subunit: Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl31Bp and has similarity to rat L31 ribosomal protein; associates with the karyopherin Sxm1p	2	S*
RPL40A	YIL153W	Ribosomal Protein of the Large subunit: Fusion protein, identical to Rpl40Bp, that is cleaved to yield ubiquitin and a ribosomal protein of the large (60S) ribosomal subunit with similarity to rat L40; ubiquitin may facilitate assembly of the ribosomal protein into ribosomes; Also known as: CEP52A	1	
RPN4	YDL020C	Regulatory Particle Non-ATPase: Transcription factor that stimulates expression of proteasome genes; Rpn4p levels are in turn regulated by the 26S proteasome in a negative feedback control mechanism; RPN4 is transcriptionally regulated by various stress responses; SGD after 20 generations, MGD after 60 generations, MGD on NaCl	2	
RPN10	YHR200W	Regulatory Particle Non-ATPase: homolog of the mammalian S5a protein, component of 26S proteasome	3	

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RPS19A	YOL121C	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps19Bp and has similarity to rat S19 ribosomal protein	1	SSS, SSS*
RPS24A	YER074W	Protein component of the small (40S) ribosomal subunit; identical to Rps24Bp and has similarity to rat S24 ribosomal protein	3	
RPS27B	YHR021C	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps27Ap and has similarity to rat S27 ribosomal protein	3	
RRD1	YIL153W	Resistant to Rapamycin Deletion: synthetic lethal with RRD2; lethality of rrd1rrd2 suppressed by increased osmolarity and also under oxygen-limited conditions	2	
RRD2	YPL152W	Resistant to Rapamycin Deletion 2: similar to hosphotyrosyl phosphatase activator (PTPA) from several organisms	2	
RRF1* (KIM4, FIL1)	YHR038W	Ribosomal Recycling Factor 1: originally characterized as FIL1, a Factor for Isocitrate Lyase expression; mitochondrial ribosome recycling factor; MGD after 20 & 60 generations, MGD on Nacl	2	
RRN10	YBL025W	Regulation of RNA polymerase I: subunit of UAF (upstream activation factor); involved in promoting high level transcription of rDNA; Upstream activation factor subunit; MGD after20 generations, SGD after 60 generations, MGD on NaCl	2	
RSC1*	YGR056W	Remodel the Structure of Chromatin: RSC1 is a member of RSC complex, which remodels the structure of chromatin; Member of RSC complex	3	
RSC2*	YLR357W	Remodel the Structure of Chromatin: Subunit of the RSC complex, which remodels the structure of chromatin; also essential for the 2-micron plasmid to overcome maternal inheritance bias	1	
RSM7*	YJR113c	Ribosomal Small subunit of Mitochondria: Mitochondrial ribosomal protein of the small subunit, has similarity to E. coli S7 ribosomal protein	1, 3	
RSM18	YER050C	Mitochondrial ribosomal protein of the small subunit, has similarity to E. coli S18 ribosomal protein	2, 3	SS
RSM19	YNR037C	Mitochondrial ribosomal protein of the small subunit, has similarity to E. coli S19 ribosomal protein; MGD after 20 & 60 generations, SGD on NaCl	2	
RSM23	YGL129C	Ribosomal Small subunit of Mitochondria: Mitochondrial ribosomal protein of the small subunit, has similarity to mammalian apoptosis mediator proteins; null mutation prevents induction of apoptosis by overproduction of metacaspase Mca1p; SGD after 20 & 60 generations, MGD on NaCl	1, 3	SSS
RSM24	YDR175C	Mitochondrial ribosomal protein of the small subunit	2, 2	S; SS*
RSM25	YIL093C	Mitochondrial ribosomal protein of the small subunit	2, 3	
RTS1	YOR014W	B-type regulatory subunit of protein phosphatase 2A (PP2A)	3	
RTT101	YJL047C	Cullin family member; subunit of a complex containing ubiquitin ligase activity; binds HRT1 and is modified by the ubiquitin like protein, RUB1; Regulator of Ty1 Transposition; Regulator of Ty1 Transposition	2	
RTT107	YHR154W	Regulator of Ty1 Transposition; Establishes Silent Chromatin; involved in silencing	3	
RTT109	YLL002W	Regulator of Ty1 Transposition; Regulation of mitochondrial network; Killed in Mutagen, sensitive to diepoxybutane and/or mitomycin C; diepoxybutane and mitomycin C resistance	2	
RUD3*	YOR216C	Golgi matrix protein that is involved in the structural organization of the cis-Golgi	3	
RVS161*	YCR009C	BAR adaptor protein, subunit of a complex (Rvs161p, Rvs167p) that regulates actin, endocytosis, and viability following starvation or osmotic stress GO Annotations	3	
RVS167*	YDR388W	BAR adaptor protein, subunit of a complex (Rvs161p-Rvs167p) that regulates actin, endocytosis, and viability following starvation or osmotic stress	2	
SAC6*	YDR129C	Fimbrin, actin-bundling protein; cooperates with Scp1p (calponin/transgelin) in the organization and maintenance of the actin cytoskeleton	3	
SAC7	YDR389W	GTPase activating protein (GAP) for Rho1p, involved in signaling to the actin cytoskeleton, null mutations suppress tor2 mutations and temperature sensitive mutations in actin; potential Cdc28p substrate	2	
SAM37*	YMR060C	Component of the mitochondrial outer membrane sorting and assembly machinery (SAM) complex; required for the sorting of some proteins to the outer membrane after import by the TOM complex (formerly TOM37)	3	
SAP155	YFR040W	Sit4 Associated Protein: Protein that forms a complex with the Sit4p protein phosphatase and is required for its function; member of a family of similar proteins including Sap4p, Sap185p, and Sap190p	3	
SAP185	YJL098W	Sit4 Associated Protein: Protein that forms a complex with the Sit4p protein phosphatase and is required for its function; member of a family of similar proteins including Sap4p, Sap155p, and Sap190p	2	
SBE2	YDR351W	Suppressor of BEm4: Protein required for bud growth	2	
SBE22	YHR103W	functionally redundant and similar in structure to SBE2; involved in bud growth	3	
SCJ1	YMR214W	dnaJ homolog	3	
SEC22	YLR268W	SECretory: Identified in a screen for dense cells that accumulated invertase at the non-permissive temperature, SEC22 encodes a v-SNARE present on ER to Golgi vesicles and is involved in anterograde and retrograde transport between the ER and Golgi; Synaptobrevin (v-SNARE) homolog	3	
SEC66	YBR171W	SECretory: Non-essential subunit of Sec63 complex (Sec63p, Sec62p, Sec66p and Sec72p); with Sec61 complex, Kar2p/BiP and Lhs1p forms a channel competent for SRP-dependent and post-translational SRP-independent protein targeting and import into the ER; SGD after20 & 60 generations, MGD on NaCl	3	
SEC72	YLR292C	SECretory: protein involved in membrane protein insertion into the ER	2	
SED1	YDR077W	Suppressor of Erd2 Deletion: Isolated as a suppressor of an erd2 deletion mutant (ERD2 is the HDEL receptor that sorts ER proteins), SED1 encodes a cell wall protein.	2	
SED4	YCR067C	Suppressor of Erd2 Deletion: Sed4p is an integral ER membrane protein, which, along along with its close homolog, Sec12p, is involved in vesicle formation at the ER; Intracellular transport protein; MGD after 20 & 60 generations, SGD on NaCl	1	
SER2	YGR208W	phosphoserine phosphatase	2	
SET5	YHR207C	ORF, Verified: SET domain-containing	3	
SFP1*	YLR403W	Inhibits nuclear protein localization when present in multiple copies; split zinc finger protein	3, 3	S, SS*
SGF11	YPL047W	11kDa subunit of the SAGA histone acetyltransferase complex involved in regulation of transcription of a subset of SAGA-regulated genes	2	
SGF29	YCL010C	SaGa associated Factor 29kDa; Probable 29kKDa Subunit of SAGA histone acetyltransferase complex	2	
SGF73	YGL066W	SaGa associated Factor 73kDa; Probable 73KkDa Subunit of SAGA histone acetyltransferase complex	2	SSS
SHE1*	YBL031W	Cytoskeletal protein of unknown function; overexpression causes growth arrest	1	SS, SS*
SHE4	YOR035C	Protein containing a UCS (UNC-45/CRO1/SHE4) domain, binds to myosin motor domains to regulate myosin function; involved in endocytosis, polarization of the actin cytoskeleton, and asymmetric mRNA localization	2	
SHE9	YDR393W	Mitochondrial inner membrane protein required for normal mitochondrial morphology, may be involved in fission of the inner membrane; forms a homo-oligomeric complex	2	
SHP1	YBL058W	UBX (ubiquitin regulatory X) domain-containing protein that regulates Glc7p phosphatase activity and interacts with Cdc48p; interacts with ubiquitylated proteins in vivo and is required for degradation of a ubiquitylated model substrate	3, 3	

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SHS1	YDL225W	Seventh Homolog of Septin: Component of the septin ring of the mother-bud neck that is required for cytokinesis; septins recruit proteins to the neck and can act as a barrier to diffusion at the membrane, and they comprise the 10nm filaments seen with EM	1	SS, SS*
SIC1	YLR079W	Substrate/Subunit Inhibitor of Cyclin-dependent protein kinase: P40 inhibitor of Cdc28p-Clb5p protein kinase complex	3	
SIT4	YDL047W	Sporulation-Induced Transcript: Type 2A-related serine-threonine phosphatase that functions in the G1/S transition of the mitotic cycle; cytoplasmic and nuclear protein that modulates functions mediated by Pkc1p including cell wall and actin cytoskeleton organization; SGD after 20 & 60 generations, MGD on NaCl	2	
SIW14	YNL032W	Synthetic Interaction with Whi2: Tyrosine phosphatase that plays a role in actin filament organization and endocytosis; localized to the cytoplasm; MGD after 20 & 60 generations, MGD on NaCl	2	
SKG1	YKR100C	Suppressor of lethality of Kex2 Gas1 double null mutant. Protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cell periphery and bud	1	
SLA1	YBL007C	Cytoskeletal protein binding protein required for assembly of the cortical actin cytoskeleton; contains 3 SH3 domains; interacts with proteins regulating actin dynamics and with proteins required for endocytosis	3, 3	
SLG1	YOR008C	Protein involved in cell wall integrity and stress response	3	
SLK19	YOR195W	Synthetic Lethal Kar3p: Kinetochore-associated protein required for normal segregation of chromosomes in meiosis and mitosis; component of the FEAR regulatory network, which promotes Cdc14p release from the nucleolus during anaphase; potential Cdc28p substrate	2	
SLM2	YNL047C	Synthetic Lethal with Mss4: Phosphoinositide PI4,5P(2) binding protein, forms a complex with Slm1p; acts downstream of Mss4p in a pathway regulating actin cytoskeleton organization in response to stress; phosphorylated by the Tor2p-containing complex TORC2; MGD after 20 & 60 generations, MGD on NaCl	1	
SLM4	YBR077C	Synthetic Lethal with Mss4: Protein with a potential role in actin cytoskeleton organization, possible component of the TOR nutrient signaling pathway; gene exhibits synthetic genetic interaction with MSS4 encoding phosphatidylinositol 4-phosphate kinase; MGD after20 generations	1	
SLM5	YCR024C	Mitochondrial asparaginyl-tRNA synthetase	3	
SLS1	YLR139C	Mitochondrial membrane protein required for assembly of respiratory-chain enzyme complexes III and IV; coordinates expression of mitochondrially-encoded genes; may facilitate delivery of mRNA to membrane-bound translation machinery	3, 2	
SLT2	YHR030C	Suppressor of lyt2; serine/threonine MAP kinase	2	
SMA1	YPL027W	Spore Membrane Assembly: MGD after 20 & 60 generations, MGD on NaCl	1	
SMF2	YHR050W	Divalent metal ion transporter	3	
SMI1*	YGR229C	Protein involved in (1,3)-beta-glucan synthesis, possibly through regulation of cell wall glucan and chitin synthesis; chromatin binding protein; 57 kDa nuclear protein	3	
SNF1	YDR477W	Sucrose NonFermenting: Protein serine/threonine kinase, required for release from glucose repression, invertase expression, sporulation, and for expression of catabolite-repressed genes when glucose is limiting; regulates Adr1p-dependent transcription primarily at the level of chromatin binding; SGD after 20 & 60 generations	2	SS*
SNF2	YOR290C	Sucrose NonFermenting: involved in the coordinate regulation of phospholipid synthesis; transcriptional regulator	3	
SNF3	YDL194W	Sucrose NonFermenting: glucose sensor; SGD after 20 & 60 generations, MGD on NaCl	2	
SNF4	YGL115W	Sucrose NonFermenting: involved in release from glucose repression, invertase expression, and sporulation; associates with Snf1p	2	
SNF5	YBR289W	Sucrose NonFermenting: Involved in global regulation of transcription; subunit of the chromatin remodeling Snf/Swi complex	2, 3	SS*
SNF6	YHL025W	Sucrose NonFermenting: Involved in global regulation of transcription; subunit of the chromatin remodeling Snf/Swi complex	3	
SNF7	YLR025W	Sucrose NonFermenting: Involved in derepression of SUC2 in response to glucose limitation; involved in glucose derepression	3	
SNF8	YPL002C	Component of the ESCRT-II complex, which is involved in ubiquitin-dependent sorting of proteins into the endosome; appears to be functionally related to SNF7; involved in glucose derepression	3	
SOD1	YJR104C	Cu, Zn superoxide dismutase; some mutations are analogous to those that cause ALS (amyotrophic lateral sclerosis) in humans	1	
SOD2	YHR008C	Manganese-containing superoxide dismutase; SGD after 20 generations, MGD after 60 generations, SGD on NaCl	2, 2	
SOH1	YGL127C	Suppressor Of Hpr1: Protein with sequence similarity to RNA polymerases; interacts with a DNA repair protein, Rad5p, in the two-hybrid system; may provide a link between recombination in direct repeats and transcription; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2	
SOY1	YBR194W	Synthetic with Old Yellow Enzyme	3	
SPA2	YLL021W	Component of the polarisome, which functions in actin cytoskeletal organization during polarized growth; acts as a scaffold for Mkk1p and Mpk1p cell wall integrity signaling components; potential Cdc28p substrate	2	
SPF1	YEL031W	Sensitivity to a killer toxin (SMK toxin) produced by Pichia Farinosa; P-type ATPase	2	
SPT2	YER161C	SuPpressor of Ty: Protein involved in negative regulation of transcription, exhibits regulated interactions with both histones and SWI-SNF components, has similarity to mammalian HMG1 proteins; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1	
SPT7	YBR081C	SuPpressor of Ty: Subunit of the SAGA transcriptional regulatory complex, involved in proper assembly of the complex; also present as a C-terminally truncated form in the SLIK/SALSA transcriptional regulatory complex	3	
SPT10*	YJL127C	SuPpressor of Ty: Putative histone acetylase, required for transcriptional regulation at core promoters, functions at or near the TATA box	3	
SPT20*	YOL148C	SuPpressor of Ty: Subunit of the SAGA transcriptional regulatory complex, involved in maintaining the integrity of the complex	3	
SRB2	YHR041C	RNA polymerase II holoenzyme/mediator subunit	3	
SRB5*	YGR104C	subunit of RNA polymerase II holoenzyme/mediator complex	3	
SRF1	YGL218W	Stress Resistance during Fermentation: ORF, Dubious	2, 3	SSS
SRF4	YDL023C	Stress Resistance during Fermentation: Dubious open reading frame, unlikely to encode a protein; not conserved in closely related Saccharomyces species; 80% of ORF overlaps the verified gene GPD1; deletion in cyr1 mutant results in loss of stress resistance	2	
SRN2	YLR119W	Component of the ESCRT-I complex, which is involved in ubiquitin-dependent sorting of proteins into the endosome; suppressor of rna1-1 mutation; may be involved in RNA export from nucleus	2	
SSD1	YDR293C	Protein with a role in maintenance of cellular integrity, interacts with components of the TOR pathway; ssd1 mutant of a clinical S. cerevisiae strain displays elevated virulence	2	
SSK1	YLR006C	Suppressor of Sensor Kinase: Cytoplasmic response regulator, part of a two-component signal transducer that mediates osmosensing via a phosphorelay mechanism; dephosphorylated form is degraded by the ubiquitin-proteasome system; potential Cdc28p substrate	3	
SSK2	YNR031C	Suppressor of Sensor Kinase: Suppressor of Sensor Kinase (SLN1); A MAP kinase kinase kinase; activator of Pbs2p; MGD after 20 & 60 generations, MGD on NaCl	1	

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SSN8	YNL025C	Suppressor of snf1: Component of RNA polymerase II holoenzyme, involved in RNA pol II carboxy-terminal domain phosphorylation, Also known as: CycC; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2	
SSO1	YPL232W	SSO1 and SSO2 encode syntaxin homologs (post-Golgi t-SNAREs); act in late stages of secretion; post-Golgi t-SNARE	1	
SSQ1	YLR369W	HSP70 family chaperone: Mitochondrial hsp70-type molecular chaperone, involved in the synthesis and assembly of iron/sulfur clusters into proteins	3, 3	SSS, SSS*
STB1	YNL309W	Sin Three Binding protein: Protein with a role in regulation of MBF-specific transcription at Start, phosphorylated by Cln-Cdc28p kinases in vitro; unphosphorylated form binds Swi6p and binding is required for Stb1p function; expression is cell-cycle regulated; MGD after 60 generations	2	
STE20	YHL007C	Signal transducing kinase of the PAK (p21-activated kinase) family, involved in pheromone response and pseudohyphal/invasive growth pathways, activated by Cdc42p; binds Ste4p at a GBB motif present in noncatalytic domains of PAK kinases	3	
STP1	YDR463W	Species-specific tRNA Processing: Transcription factor, activated by proteolytic processing in response to signals from the SPS sensor system for external amino acids; activates transcription of amino acid permease genes and may have a role in tRNA processing; SGD after 20 & 60 generations, SGD on NaCl	2	SSS
STP22	YCL008C	Component of the ESCRT-I complex, which is involved in ubiquitin-dependent sorting of proteins into the endosome; homologous to the mouse and human Tsg101 tumor susceptibility gene; mutants exhibit a Class E Vps phenotype	3	
SUB1	YMR039C	Suppressor of TFIIB mutations; transcriptional coactivator	2	
SUM1	YDR310C	Suppresor of mar1-1 (sir2) mutation; nuclear protein involved in silencing	2	
SUR2	YDR297W	Sphingosine hydroxylase; has a role in sphingolipid metabolism	2	
SUV3	YPL029W	SUppressor of var1: ATP-dependent RNA helicase, component of the mitochondrial degradosome along with the RNase Msu1p; the degradosome associates with the ribosome and mediates turnover of aberrant or unprocessed RNAs	3, 2	SS*
SUT2	YPR009W	Involved in sterol uptake; homologous to SUT1	1	SS*
SVL3	YPL032C	Protein of unknown function, mutant phenotype suggests a potential role in vacuolar function; green fluorescent protein (GFP)-fusion protein localizes to the cell periphery, cytoplasm, bud, and bud neck	3	
SWA2	YDR320C	Synthetic lethal With Arf1: auxilin-like protein	2	
SWC5	YBR231C	Protein of unknown function, component of the Swr1p complex that incorporates Htz1p into chromatin	1	SS*
SWD1	YAR003W	Subunit of the COMPASS complex, which methylates histone H3 on lysine 4 and is required in transcriptional silencing near telomeres	1	SSS, SSS*
SWF1	YDR126W	Spore Wall Formation	3	
SWI3	YJL176C	SWItching deficient: transcription factor	3	
SWI4	YER111C	SWItching deficient: Involved in cell cycle dependent gene expression; transcription factor; SGD after 60 generations, MGD on NaCl	3	
SWI6	YLR182W	SWItching deficient1/17/2005 Transcription cofactor, forms complexes with DNA-binding proteins Swi4p and Mbp1p to regulate transcription at the G1/S transition; involved in meiotic gene expression; localization regulated by phosphorylation; potential Cdc28p substrate	3	
SWS2	YNL081C	Sick Without Securin: Putative mitochondrial ribosomal protein of the small subunit, has similarity to E. coli S13 ribosomal protein	3	
SYC1	YOR179C	Similar to Ysh1 C-terminal: Similar to Ysh1 C terminal: Subunit of the APT subcomplex of cleavage and polyadenylation factor, may have a role in 3' end formation of both polyadenylated and non-polyadenylated RNAs; SGD after 20 & 60 generations, MGD on NaCl	2	SSS
TAT1	YBR069C	Amino acid transport protein for valine, leucine, isoleucine, and tyrosine	1	
TCO89	YPL180W	Tor Complex One, 89 kDa subunit; Tor1p binding protein	2	
TEF4	YKL081W	Translation elongation factor EF-1gamma	2	
THI3	YDL080C	THIamine metabolism: Probable decarboxylase, required for expression of enzymes involved in thiamine biosynthesis; may have a role in catabolism of amino acids to long-chain and complex alcohols	2	SS*
THR1	YHR025W	homoserine kinase	3	
THR4	YCR053W	THReonine: threonine synthase; SGS on NaCl	2	
TKL1	YPR074C	Transketolase 1	2	
TLG2	YOL018C	member of the syntaxin family of t-SNAREs; tSNARE that affects a Late Golgi compartment	3	
TOM7	YNL070W	Translocase of the Outer Membrane: Involved in mitochondrial protein import; translocase of the outer mitochondrial membrane, MGD after 20 & 60 generations, MGD on NaCl	2	
TOP3	YLR234W	DNA Topoisomerase III	2, 3	
TPP1	YMR156C	Three Prime Phosphatase: DNA 3'-phosphatase that functions in the repair of endogenous DNA damage; functionally redundant with Apn1p and Apn2p	1	SSS, SSS*
TPS1*	YBR126C	Trehalose-6-Phosphate Synthase: Probable regulator of glucose influx into the cell & into glycolytic pathway, indirectly regulating glucose-induced signalling (activation & inactivation) & initial step(s) of glucose metabolism. Homologue of E. coli otsA protein; 56 kD synthase subunit of trehalose-6-phosphate synthase/phosphatase complex; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1	
TPS2*	YDR358C	Trehalose-6-phosphate phosphatase: SGD after 20 & 60 generations, MGD on NaCl	2	
TRF5	YNL299W	DNA polymerase sigma: TRF4 homolog; TRF4/5 function is required for proper mitosis; exhibits homology to Trf4p and Top1p	2	
TRP1	YDR070W	TRyPtophan requiring: Phosphoribosylanthranilate isomerase that catalyzes the third step in tryptophan biosynthesis, MGD after 20 & 60 generations, SGD on NaCl	2	
TRP5	YGL026G	tryptophan synthetase	1	SSS, SSS*
TSR2*	YLR435W	Twenty S rRNA accumulation: Protein with a potential role in pre-rRNA processing	2, 2	
TUF1	YOR187W	translation elongation factor Tu, mitochondrial: Mitochondrial translation elongation factor Tu; comprises both GTPase and guanine nucleotide exchange factor activities, while these activities are found in separate proteins in S. pombe and humans	2, 3	SSS
TUP1*	YCR084C	deoxyThymidine monophosphate Uptake: General repressor of transcription (with Cyc8p), mediates glucose repression; exhibits similarity to beta subunits of G proteins	3, 3	
UBR2	YLR024C	Cytoplasmic ubiquitin-protein ligase (E3)	2	
UBP6	YFR010W	UBiquitin-specific Protease: Ubiquitin-specific protease situated in the base subcomplex of the 26S proteasome, releases free ubiquitin from branched polyubiquitin chains; deletion causes hypersensitivity to cycloheximide and other toxic compounds; MGD after 20 generations, SGD after 60 generations, SGD on NaCl	2	SSS, SS
UGA2	YBR006W	Utilization of GABA: involved in utilization of GABA as a nitrogen source; succinate semialdehyde dehydrogenase; MGD after20 & 60 generations; MGD on NaCl	1	

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UGO1	YDR470C	UGO (Japanese for fusion): Protein of unknown function; outer membrane component of the mitochondrial fusion machinery; Ugo1p bind directly to Fzo1p and Mgm1p and thereby link these two GTPases during mitochondrial fusion; MGD after 20 & 60 generations, MGD on NaCl	2	SSS*
UMP1	YBR173C	Ubiquitin-Mediated Proteolysis: Involved in ubiquitin-mediated proteolysis; 20S proteasome maturation factor	2	
URE2	YNL229C	glutathione transferase (putative): Nitrogen catabolite repression regulator that acts by inhibition of GLN3 transcription in good nitrogen source; altered form of Ure2p creates [URE3] prion	2	
VAC7	YNL054W	VACuolar segregation: Integral vacuolar membrane protein; may function to regulate Fab1p kinase activity	1	
VAC14	YLR386W	VACuole morphology and inheritance mutant: Activator of Fab1p; essential for the regulated synthesis of PtdIns(3,5)P(2), for control of trafficking of some proteins to the vacuole lumen via the MVB, and for maintenance of vacuole size and acidity	3	
VAM6	YDL077C	VAcuolar Morphogenesis: Vacuolar protein that plays a critical role in the tethering steps of vacuolar membrane fusion by facilitating guanine nucleotide exchange on small guanosine triphosphatase Ypt7p	2	SS
VAM7	YGL212W	VAcuolar Morphogenesis: Regulator of vacuolar morphogenesis; hydrophilic protein, heptad repeat motif	1	SS
VAM10	YOR068C	[Abnormal]Vacuole Morphology: Required for normal tethering of vacuoles prior to fusion.	1	
VAN1	YML115C	VANadate resistance protein: Mannosyltransferase with a role in protein N-glycosylation, MGD after 20 generations, MGD on NaCl	3	
VID21*	YDR359C	Component of the NuA4 histone acetyltransferase complex	2	
VID22	YLR373C	Vacuole import and degradation	3	
VMA2	YBR127C	Vacuolar Membrane Atpase: Vacuolar H+ ATPase regulatory subunit (subunit B) of the catalytic (V1) sector, Also known as: ATPSV; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2, 3	
VMA4	YOR332W	vacuolar ATPase V1 domain subunit E (27 kDa)	1, 2	S, SSS*
VMA5	YKL080W	Vacuolar H+ ATPase subunit C of the catalytic (V1) sector	3	
VMA7*	YGR020C	Vacuolar H+ ATPase subunit F of the catalytic (V1) sector	2	SSS
VMA8	YEL051W	vacuolar ATPase V1 domain subunit D	2	
VMA10	YHR039C-B	vacuolar ATPase V1 domain subunit G (13 kDa): Vacuolar H+ ATPase subunit G of the catalytic (V1) sector	2	
VPS1	YKR001C	Vacuolar Protein Sorting: involved in vacuolar protein sorting and normal organization of intracellular membranes; probably required for membrane-protein retention in a late Golgi compartment; putative GTP-binding protein; similar to mammalian Mx proteins	3	
VPS3	YDR495C	Vacuolar Protein Sorting	3	SSS
VPS4	YPR173C	Defective in vacuolar protein sorting; homologous to mouse SKD1 and to human hVPS4; AAA-type ATPase	2	
VPS5	YOR069W	Component of the retromer coat that retrieves proteins from late endosomes; sorting nexin 1 homolog	1	
VPS8	YAL002W	involved in vacuolar protein sorting; required for localization and trafficking of the CPY sorting receptor; Vps8p is a membrane-associated hydrophilic protein which contains a C-terminal cysteine-rich region that conforms to the H2 variant of the RING finger Zn2+ binding motif.	2	
VPS9	YML097C	Protein required for Golgi to vacuole trafficking, has similarity with mammalian ras inhibitors	3	
VPS13	YLL040C	homologous to human COH1; component of peripheral vacuolar membrane protein complex	2	
VPS16	YPL045W	Vacuolar sorting protein	3, 3	SSS
VPS20	YMR077C	vaculolar protein sorting (putative)	3	
VPS25	YJR102C	Component of the ESCRT-II complex, which is involved in ubiquitin-dependent sorting of proteins into the endosome	3	
VPS27	YNR006W	Vacuolar Protein Sorting: hydrophilic protein; has cysteine rich putative zinc finger essential for function	2	
VPS28	YPL065W	Component of the ESCRT-I complex, which is involved in ubiquitin-dependent sorting of proteins into the endosome; involved in transport of precursors for soluble vauolar hydrolases from the late endosome to the vacuole	3	
VPS29	YHR012W	Protein involved in vacuolar protein sorting	3	
VPS30	YPL120W	Protein required for sorting and delivery of soluble hydrolases to the vacuole	2	
VPS33*	YLR396C	vacuolar sorting protein essential for vacuolar morphogenesis and function; involved in vacuolar protein targeting	3	
VPS34	YLR240W	Vacuolar Protein Sorting: Phosphatidylinositol 3-kinase responsible for the synthesis of phosphatidylinositol 3-phosphate; forms membrane-associated signal transduction complex with Vps15p to regulate protein sorting; similar to p110 subunit of mammalian PI 3-kinase; SGD after 20 generations, MGD after 60 generations, MGD on NaCl	3, 3	SSS
VPS35	YJL154C	Protein involved in vacuolar sorting; retromer complex component	2	
VPS36	YLR417W	Component of the ESCRT-II complex, which is involved in ubiquitin-dependent sorting of proteins into the endosome	3	
VPS38	YLR360W	involved in vacuolar protein targeting	2	
VPS41	YDR080W	vacuolar protein sorting; component of vacuolar membrane protein complex	1	SSS, SSS*
VPS45	YGL095C	Vacuolar Protein Sorting: Protein of the Sec1p family, essential for vacuolar protein sorting; required for the function of both Pep12p and the early endosome/late Golgi SNARE Tlg2p	3	
VPS51	YKR020W	whiskey (whi) mutant; forms a tetramer with VPS52, VPS53, and VPS54	3	
VPS52	YDR484W	Vacuolar Protein Sorting: May interact with actin as a component or controller of the assembly or stability of the actin cytoskeleton; involved in localization of actin and chitin; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	3	SSS
VPS54	YDR027C	Component of the GARP (Golgi-associated retrograde protein) complex, Vps51p-Vps52p-Vps53p-Vps54p, which is required for retrograde transport to the late Golgi; potentially phosphorylated by Cdc28p	3, 3	
VPS60	YDR486C	vacuolar protein sorting: Null mutant is viable but a class E vps mutant (missorts vacuolar hydrolases and accumulates late endosomal compartment)	2	SSS*
VPS61	YDR136C	Dubious open reading frame, unlikely to encode a protein; not conserved in closely related Saccharomyces species; 4% of ORF overlaps the verified gene RGP1; deletion causes a vacuolar protein sorting defect	3	
VPS63	YLR261C	Dubious open reading frame, unlikely to encode a protein; not conserved in closely related Saccharomyces species; 98% of ORF overlaps the verified gene YPT6; deletion causes a vacuolar protein sorting defect	3	
VPS64	YDR200C	Vacuolar Protein Sorting	2	
VPS65*	YLR322W	Dubious open reading frame, unlikely to encode a protein; not conserved in closely related Saccharomyces species; 75% of ORF overlaps the verified gene SFH1; deletion causes a vacuolar protein sorting defect	3	
VPS66	YPR139C	Vacuolar Protein Sorting	3	
VPS74	YDR372C	Vacuolar Protein Sorting	3	

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VRP1	YLR337C	proline-rich protein verprolin: Involved in cytoskeletal organization and cellular growth; Proline-rich protein verprolin	3, 3	SSS, SSS*
WHI2	YOR043W	WHIskey: Protein required, with binding partner Psr1p, for full activation of the general stress response, possibly through Msn2p dephosphorylation; regulates growth during the diauxic shift; negative regulator of G1 cyclin expression	2	
XRS2*	YDR369C	X-Ray Sensitive: classified as an early recombination function, required for DNA repair but dispensable for mitotic recombination (xrs2 is hyper-Rec during vegatative growth), required for double strand breaks, meiotic recombination and spore viability; DNA repair protein	2	
YAF9*	YNL107W	Yeast homolog of the human leukemogenic protein AF9: Subunit of both the NuA4 histone H4 acetyltransferase complex and the SWR1 complex, may function to antagonize silencing near telomeres; interacts directly with Swc4p, has homology to human leukemogenic protein AF9, contains a YEATS domain	2	
YCK3	YER123W	Yeast Casein Kinase: plasma membrane-bound casein kinase I homolog; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1	
YDJ1*	YNL064C	yeast dnaJ homolog (nuclear envelope protein); heat shock protein	3	SSS
YHM1	YDL198C	Mitochondrial GTP/GDP transporter, essential for mitochondrial genome maintenance; has a role in mitochondrial iron transport; member of the mitochondrial carrier family; (putative) mitochondrial carrier protein; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	3, 3	
YIM2	YMR151W	Dubious open reading frame, unlikely to encode a protein; not conserved in closely related Saccharomyces species; 5% of ORF overlaps the verified gene IMP1	1, 2	SS*
YME1	YPR024W	Yeast Mitochondrial Escape: Mitochondrial inner membrane protease of the AAA family, responsible for degradation of unfolded or misfolded mitochondrial gene products; mutation causes an elevated rate of mitochondrial turnover; MGD after 60 generations, MGD on NaCl	3	
YND1	YER005W	Yeast Nucleoside Diphosphatase: apyrase (NDPase/NTPase)	2	
YOS9	YDR057W	Glycoprotein of the ER membrane, involved in ER-to-Golgi transport of glycosylphosphatidylinositol (GPI)-anchored proteins	2	
YPT6	YLR262C	Ras-like GTP binding protein involved in the secretory pathway, required for fusion of endosome-derived vesicles with the late Golgi; has similarity to the human GTPase, Rab6	3	
YPT31	YER031C	probably involved in intra-Golgi transport or in the formation of transport vesicles at the most distal Golgi compartment; ras-like GTPase, highly homologous to YPT32	3	
ZAP1	YJL056C	Zinc-regulated transcription factor, binds to zinc-responsive promoter elements to induce transcription of certain genes in the presence of zinc; regulates its own transcription; contains seven zinc-finger domains	3	
ZDS1	YMR273C	Zillion Different Screens: Protein that interacts with silencing proteins at the telomere, involved in transcriptional silencing; also has a role in localization of Bcy1p, which is a regulatory subunit of protein kinase A; paralog of Zds2p	3	
ZUO1*	YGR285C	zuotin, Z-DNA binding protein: Cytosolic ribosome-associated chaperone, contains a DnaJ domain; together with Ssz1p, acts as a chaperone for nascent polypeptide chains; MGD after 20 generations , SGD after 60 generations, MGD on NaCl	2	
ORF	YBL046W	ORF, Uncharacterized	1	SSS, SS*
ORF	YBL083C	ORF, Dubious	2	
ORF	YBR134W	ORF, Dubious; MGD after 20 generations, SGD on NaCl	2	
ORF	YBR300C	ORF, Dubious	2	
ORF	YCL001W-A	ORF, Uncharacterized	2	
ORF	YCR043C	ORF, Uncharacterized	2	
ORF	YDL025C	ORF, Uncharacterized: Protein of unknown function, potentially phosphorylated by Cdc28p; SGD after 20 & 60 generations, MGD on NaCl	1	
ORF	YDL032W	ORF, Dubious	2, 3	SSS
ORF	YDL068W	ORF, Dubious	2, 3	S; SS*
ORF	YDL073W	ORF, Uncharacterized	2	SS; SSS*
ORF	YDL203C	ORF, Uncharacterized	2	
ORF	YDR049W	ORF, Uncharacterized	3	
ORF	YDR065W	ORF, Uncharacterized	2, 3	
ORF	YDR114C	ORF, Dubious	1, 2	S; SS*
ORF	YDR115W	Putative mitochondrial ribosomal protein of the large subunit, has similarity to E. coli L34 ribosomal protein; required for respiratory growth, as are most mitochondrial ribosomal proteins	1, 2	S; S*
ORF*	YDR149C	ORF, Dubious	1	SSS, SSS*
ORF	YDR203W	ORF, Dubious	2	
ORF	YDR230W	ORF, Dubious	1, 3	
ORF	YDR269C	ORF, Dubious	2	
ORF	YDR332W	ORF, Uncharacterized	2, 3	
ORF	YDR360W	ORF, Dubious	2	
ORF	YDR442W	ORF, Dubious	2, 2	SSS*
ORF	YDR506C	ORF, Uncharacterized	2	SS*
ORF	YDR509W	ORF, Dubious	2, 3	S; SSS*
ORF*	YDR532C	ORF, Uncharacterized; Protein of unknown function that localizes to the nuclear side of the spindle pole body and along short spindles; forms a complex with Spc105p	2, 3	
ORF	YDR540C	ORF, Uncharacterized	2	
ORF	YEL023C	ORF, Uncharacterized	2	
ORF*	YEL033W	ORF, Dubious	2	S; SS*
ORF	YER077C	ORF, Uncharacterized	1, 2	
ORF	YER084W	ORF, Dubious	2	
ORF	YER087W	ORF, Uncharacterized	2, 3	
ORF	YER113C	ORF, Uncharacterized; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	2	
ORF	YER139C	ORF, Uncharacterized	1	
ORF	YFR020W	ORF, Dubious	2	
ORF	YGL152C	ORF, Dubious; MGD after 20 & 60 generations	1	

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ORF	YGR071C	ORF, Uncharacterized	3	
ORF	YGR102C	ORF, Uncharacterized; SGD after 20 & 60 generations, SGD on NaCl	2, 2	
ORF	YGR122W	ORF, Uncharacterized	3	
ORF	YGR150C	ORF, Uncharacterized; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1, 2	SS, SS*
ORF	YGR206W	ORF, Uncharacterized	1	S
ORF	YGR228W	ORF, Dubious	3	
ORF	YHL005C	ORF, Dubious	2, 2	
ORF	YHR009C	ORF, Uncharacterized	3	
ORF	YHR029C	ORF, Uncharacterized	3	
ORF	YHR087W	ORF, Uncharacterized	3	
ORF	YHR100C	ORF, Uncharacterized	3	
ORF	YIL029C	ORF, Uncharacterized	2	
ORF	YIL077C	ORF, Uncharacterized	2	
ORF	YIL100W	ORF, Dubious	1	
ORF	YJL022W	ORF, Dubious	3	
ORF	YJL028W	ORF, Dubious	2, 3	SSS
ORF	YJL046W	ORF, Uncharacterized; MGD after 20 generations, MGD on NaCl	2, 2	
ORF	YJL123C	ORF, Uncharacterized	3	
ORF	YJL180C	ORF, Uncharacterized	2, 2	
ORF	YJR121W	ORF, Uncharacterized	1, 2	
ORF	YKL023W	ORF, Uncharacterized	2	
ORF	YKL033W-A	ORF, Uncharacterized: Similar to S. pombe hypothetical proteins	1	SSS, SSS*
ORF	YKL037W	ORF, Uncharacterized	3	
ORF	YKL053W	ORF, Dubious	3	
ORF	YKL115C	ORF, Dubious	2	
ORF	YKL169C	ORF, Dubious	2, 2	
ORF	YLL033W	ORF, Uncharacterized	2, 2	
ORF	YLR091W	ORF, Uncharacterized; SGD after 20 & 60 generations, MGD on NaCl	2, 2	
ORF	YLR111W	ORF, Dubious	3	
ORF	YLR149C	ORF, Uncharacterized	1, 3	
ORF	YLR193C	ORF, Uncharacterized	2, 1	
ORF	YLR202C	ORF, Dubious; MGD after 20 & 60 generations, MGD on NaCl	2, 2	
ORF	YLR232W	ORF, Dubious	2, 2	
ORF*	YLR235C	ORF, Dubious: Involved in meiotic nuclear division.	2, 2	
ORF	YLR338W	ORF, Dubious	3, 3	SSS, SSS*
ORF*	YLR358C	ORF, Dubious; SGD after 20 generations, MGD after 60 generations, SGD on NaCl	1	
ORF	YLR374C	ORF, Dubious	2	
ORF	YLR426W	ORF, Uncharacterized	2	
ORF	YML090W	ORF, Dubious; MGD after 20 & 60 generations, MGD on NaCl	1, 2	
ORF	YML122C	ORF, Dubious	2	
ORF	YMR034C	ORF, Uncharacterized	3	
ORF	YMR098C	ORF, Uncharacterized	2, 2	
ORF*	YMR293C	ORF, Uncharacterized: protein similar to bacterial glutamyl-tRNA amidotransferases	1, 2	SSS*
ORF	YNL056W	ORF, Uncharacterized; MGD after 20 & 60 generations, MGD on NaCl	1	
ORF	YNL089C	ORF, Dubious; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	1	
ORF	YNL115C	ORF, Uncharacterized, MGD after 20 & 60 generations, MGD on NaCl	2	
ORF	YNL140C	ORF, Dubious; SGD after 20 & 60 generations, MGD on NaCl	3, 3	
ORF	YNL170W	ORF, Dubious	1, 2	S*
ORF	YNL184C	ORF, Dubious	2, 2	SSS*
ORF	YNL324W	ORF, Dubious	2	
ORF	YNR005C	ORF, Dubious	1	
ORF	YNR020C	ORF, Uncharacterized	2	
ORF*	YNR068C	ORF, Uncharacterized	1	
ORF	YOL007C	Appears to be a structural component of the chitin synthase 3 complex	2	
ORF	YOL008W	ORF, Uncharacterized	2	
ORF	YOL050C	ORF, Dubious	2	
ORF	YOL075C	ORF, Uncharacterized	1	
ORF	YOR051C	Nuclear protein that inhibits replication of Brome mosaic virus in S. cerevisiae, which is a model system for studying replication of positive-strand RNA viruses in their natural hosts	1	
ORF	YOR091W	ORF, Uncharacterized	2	
ORF	YOR135C	ORF, Dubious	2	
ORF	YOR164C	ORF, Uncharacterized	2	
ORF	YOR199W	ORF, Dubious	1, 2	S*

Appendix B: Zymocin sensitive diploid deletion mutants in *S. cerevisiae*

ORF	YOR200W	ORF, Dubious	**2, 2**	SS*
ORF	YOR305W	ORF, Uncharacterized	**2, 3**	SS, SSS*
ORF	YOR331C	ORF, Dubious	**2, 3**	
ORF	YOR364W	ORF, Dubious	**1, 3**	SSS
ORF*	YPL066W	ORF, Uncharacterized	**1**	SS*
ORF	YPL137C	ORF, Uncharacterized; MGD after 20 generations, SGD after 60 generations	**3, 3**	
ORF	YPL150W	ORF, Uncharacterized	**2**	
ORF	YPL158C	ORF, Uncharacterized; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	**1**	
ORF	YPL183C	ORF, Uncharacterized; MGD after 20 generations, SGD after 60 generations, MGD on NaCl	**2**	
ORF	YPL225W	ORF, Uncharacterized	**2**	
ORF	YPR004C	ORF, Uncharacterized	**1**	SS*
ORF	YPR050C	ORF, Dubious	**2**	
ORF	YPR099C	ORF, Dubious	**1, 2**	S*
ORF	YPR116W	ORF, Uncharacterized	**1, 2**	
ORF	YPR123C	ORF, Dubious	**2**	SSS*
ORF	YPR153W	ORF, Uncharacterized	**1**	S*

Gene* Gene deletion or ORF demonstrates cross sensitivity to ionizing radiation

Sensitivity: 1 = partial growth on 66% zymocin plate, partial or uninhibited growth on 33% zymocin plate
2 = no growth on 66% zymocin plate, partial or uninhibited growth on 33% zymocin plate
3 = no growth on 66% or 33% zymocin plate

Dilution Plating:

S = 5 fold enhanced sensitivity (compared to WT) following dilution plating on 25 or 33% zymocin plate
SS = 25 fold enhanced sensitivity following dilution plating on 25 or 33% zymocin plate
SSS = 125 fold (or greater) enhanced sensitivity following dilution plating on 25 or 33% zymocin plate

S*, SS*, SSS* enhanced sensitivity of mutant determined on 66% zymocin plate

Appendix C: Zymocin resistant diploid deletion mutants in *S. cerevisiae*

ORF	Gene	Function	Zymocin Resistance	Dilution Plating
YJR105W	ADO1*	adenosine kinase	3	R
YJR058C	APS2	Related to the sigma subunit of the mammalian plasma membrane clathrin-associated protein (AP-2) complex; Clathrin-associated protein, small subunit	2	R
YDR441C	APT2	Adenine PhosphoribosylTransferase: Apparent pseudogene, not transcribed or translated under normal conditions; encodes a protein with similarity to adenine phosphoribosyltransferase, but artificially expressed protein exhibits no enzymatic activity	2	R
YDR101C	ARX1	ORF, Verified	2	RR
YAL020C	ATS1	Protein with a potential role in regulatory interactions between microtubules and the cell cycle, as suggested by genetic and physical interactions with Nap1p and genetic interactions with TUB1	3	RRR*
YJL188C	BUD19*	ORF, Dubious: Dubious open reading frame, unlikely to encode a protein; not conserved in closely related Saccharomyces species; 88% of ORF overlaps the verified gene **RPL39**; diploid mutant displays a weak budding pattern phenotype in a systematic assay	3	R
YLR062C	BUD28	Dubious open reading frame, unlikely to encode a protein; not conserved in closely related Saccharomyces species; 98% of ORF overlaps the verified gene **RPL22A**; diploid mutant displays a weak budding pattern phenotype in a systematic assay	3	RR
YBR023C	CHS3	Chitin synthase III, catalyzes the transfer of N-acetylglucosamine (GlcNAc) to chitin; required for synthesis of the majority of cell wall chitin, the chitin ring during bud emergence, and spore wall chitosan	3	RRR*
YHR142W	CHS7	Protein of unknown function, involved in chitin biosynthesis by regulating Chs3p export from the ER	3	RRR*
YLR216C	CPR6	Peptidyl-prolyl cis-trans isomerase (cyclophilin), catalyzes the cis-trans isomerization of peptide bonds N-terminal to proline residues; binds to Hsp82p and contributes to chaperone activity	1	R
YGR155W	CYS4	Cystathionine beta-synthase, catalyzes the first committed step of transsulfuration in cysteine biosynthesis	3	R
YGL078C	DBP3	Putative ATP-dependent RNA helicase of the DEAD-box family involved in ribosomal biogenesis	3	R
YGR200C	ELP2	Elongator protein, part of the six-subunit RNA polymerase II Elongator histone acetyltransferase complex; target of Kluyveromyces lactis zymocin	2	RRR*
YPL086C	ELP3	Histone acetyltransferase subunit of the Elongator complex, which is a component of the RNA polymerase II holoenzyme; activity is directed specifically towards histones H3 and H4; disruption confers resistance to K. lactis zymotoxin	2	RRR*
YMR312W	ELP6	Elongator protein, part of the HAP subcomplex of Elongator, which is a six-subunit component of the RNA polymerase II holoenzyme; required for Elongator structural integrity and histone acetyltransferase activity	3	RRR*
YKR027W	FMP50	Found in Mitochondrial Proteome	2	R
YGL195W	GCN1	Positive regulator of the Gcn2p kinase activity, forms a complex with Gcn20p; proposed to stimulate Gcn2p activation by an uncharged tRNA	3	R
YKR026C	GCN3	Alpha subunit of the translation initiation factor eIF2B, the guanine-nucleotide exchange factor for eIF2; activity subsequently regulated by phosphorylated eIF2; first identified as a positive regulator of GCN4 expression	2	RR
YDL035C	GPR1	Plasma membrane G-protein coupled receptor that interacts with the heterotrimeric G protein alpha subunit, Gpa2p, and with Plc1p; sensor that integrates nutritional signals with the modulation of cell fate via PKA and cAMP synthesis	2	R, R
YNL031C	HHT2	One of two identical histone H3 proteins (see also HHT1); core histone required for chromatin assembly, involved in heterochromatin-mediated telomeric and HM silencing; regulated by acetylation, methylation, and mitotic phosphorylation	2	R
YDR174W	HMO1	High MObility group (HMG) family: Chromatin associated high mobility group (HMG) family member involved in genome maintenance; rDNA-binding component of the Pol I transcription system; associates with a 5'-3' DNA helicase and Fpr1p, a prolyl isomerase	3	R
YDR072C	IPT1	Inositolphosphotransferase 1, involved in synthesis of mannose-(inositol-P)2-ceramide (M(IP)2C), which is the most abundant sphingolipid in cells, mutation confers resistance to the antifungals syringomycin E and DmAMP1 in some growth media	3	RRR*
YNL227C	JJJ1	Protein that may function as a cochaperone, as suggested by the presence of a DnaJ-like domain	3	RRR
YPL125W	KAP120	KAryoPherin: Karyopherin with a role in the assembly or export of 60S ribosomal subunits	2	RRR
YGL203C	KEX1	Protease involved in the processing of killer toxin and alpha factor precursor; cleaves Lys and Arg residues from the C-terminus of peptides and proteins	3	RRR
YKL110C	KTI12	Protein associtiated with the RNA polymerase II Elongator complex; involved in sensitivity to G1 arrest induced by Kluyveromyces lactis toxin, zymocin	3	RRR*
YFR001W	LOC1*	Nuclear protein involved in asymmetric localization of ASH1 mRNA; binds double-stranded RNA in vitro	3	RRR
YDR439W	LRS4	Loss of RDNA Silencing: Protein involved in rDNA silencing; positively charged coiled-coil protein with limited similarity to myosin	1	R, R

Appendix C: Zymocin resistant diploid deletion mutants in *S. cerevisiae*

YGR289C	MAL11	Maltose permease, inducible high-affinity maltose transporter (alpha-glucoside transporter); encoded in the MAL1 complex locus; member of the 12 transmembrane domain superfamily of sugar transporters	2	RR
YNL142W	MEP2	Ammonia permease; belongs to a ubiquitous family of cytoplasmic membrane proteins that transport only ammonium (NH4+)	2	R
YML062C	MFT1	Protein involved in mitochondrial import of fusion proteins; mitochondrial targeting protein	2	R
YKL009W	MRT4*	Protein involved in mRNA turnover and ribosome assembly, localizes to the nucleolus	3	RRR
YDR140W	MTQ2	Putative S-adenosylmethionine-dependent methyltransferase of the seven beta-strand family	3	RR
YDL040C	NAT1*	N-terminal AcetylTransferase: Subunit of the N-terminal acetyltransferase NatA (Nat1p, Ard1p, Nat5p); N-terminally acetylates many proteins, which influences multiple processes such as the cell cycle, heat-shock resistance, mating, sporulation, and telomeric silencing	2	RR
YNL119W	NCS2	Protein with a role in urmylation and in invasive and pseudohyphal growth; inhibits replication of Brome mosaic virus in S. cerevisiae, which is a model system for studying replication of positive-strand RNA viruses in their natural hosts	3	R
YGL211W	NCS6	Protein with a role in urmylation and in invasive and pseudohyphal growth; inhibits replication of Brome mosaic virus in S. cerevisiae, which is a model system for studying replication of positive-strand RNA viruses in their natural hosts	2	R
YIL038C	NOT3	Subunit of the CCR4-NOT complex, which is a global transcriptional regulator with roles in transcription initiation and elongation and in mRNA degradation	2	R
YGR159C	NSR1	Nucleolar protein that binds nuclear localization sequences, required for pre-rRNA processing and ribosome biogenesis	1	R
YGL151W	NUT1	Component of the RNA polymerase II mediator complex, which is required for transcriptional activation and also has a role in basal transcription	1	R
YGR178c	PBP1	Poly(A)-binding protein binding protein; interacts with poly(A)-binding protein	2	RR
YIL117C	PRM5	Pheromone-regulated protein, predicted to have 1 transmembrane segment; induced during cell integrity signalling	3	RR
YJR059W	PTK2	Putative serine/threonine protein kinase involved in regulation of ion transport across plasma membrane; enhances spermine uptake	2	RRR
YGL246C	RAI1*	Nuclear protein that binds to and stabilizes the exoribonuclease Rat1p, required for pre-rRNA processing	2	R
YBR267W	REI1	Protein of unknown function involved in bud growth in the mitotic signaling network; proposed negative regulator of Swe1p and Gin4p; contains dispersed C2H2 zinc finger domains	2	R
YNL139C	RLR1*	Required for LacZ RNA expression from certain plasmids; suppressor of the Transcriptional (T) defect of Hpr1 (H) by Overexpression (O); plays a role in transcription elongation by RNA polymerase II; Involved in transcription	2	R
YDL001W	RMD1	Required for Meiotic nuclear Division: Cytoplasmic protein required for sporulation	2	RR
YJR063W	RPA12	RNA polymerase I subunit A12.2; contains two zinc binding domains, and the N terminal domain is responsible for anchoring to the RNA pol I complex Also known as: A12.2	1	RR
YNL248C	RPA49	RNA polymerase I subunit A49	3	R
YBR031W	RPL4A	N-terminally acetylated protein component of the large (60S) ribosomal subunit, nearly identical to Rpl4Bp and has similarity to E. coli L4 and rat L4 ribosomal proteins	3	R
YLR448W	RPL6B	Protein component of the large (60S) ribosomal subunit, has similarity to Rpl6Bp and to rat L6 ribosomal protein; binds to 5.8S rRNA	3	R
YHL033C	RPL8A	Ribosomal protein L4 of the large (60S) ribosomal subunit, nearly identical to Rpl8Bp and has similarity to rat L7a ribosomal protein; mutation results in decreased amounts of free 60S subunits	3	R
YNL067W	RPL9B	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl9Ap and has similarity to E. coli L6 and rat L9 ribosomal proteins	1	R
YGR085C	RPL11B	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl11Ap; involved in ribosomal assembly; depletion causes degradation of proteins and RNA of the 60S subunit; has similarity to E. coli L5 and rat L11	3	R
YDL082W	RPL13A	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl13Bp; not essential for viability; has similarity to rat L13 ribosomal protein	1	RRR
YGR155W	RPL17B	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl17Ap and has similarity to E. coli L22 and rat L17 ribosomal proteins	3	R
YBR084C-A	RPL19A	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl19Bp and has similarity to rat L19 ribosomal protein; rpl19a and rpl19b single null mutations result in slow growth, while the double null mutation is lethal	3	R
YBL027W	RPL19B	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl19Bp and has similarity to rat L19 ribosomal protein; rpl19a and rpl19b single null mutations result in slow growth, while the double null mutation is lethal	3	RR
YBR191W	RPL21A	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl21Bp and has similarity to rat L21 ribosomal protein	3	R
YLR061W	RPL22A	Protein component of the large (60S) ribosomal subunit, has similarity to Rpl22Bp and to rat L22 ribosomal protein	3	RR

Appendix C: Zymocin resistant diploid deletion mutants in *S. cerevisiae*

YGL031C	RPL24A	Ribosomal protein L30 of the large (60S) ribosomal subunit, nearly identical to **Rpl24Bp** and has similarity to rat L24 ribosomal protein; not essential for translation but may be required for normal translation rate	**3**	**R**
YGR148C	RPL24B	Ribosomal protein L30 of the large (60S) ribosomal subunit, nearly identical to **Rpl24Ap** and has similarity to rat L24 ribosomal protein; not essential for translation but may be required for normal translation rate	**2**	**R**
YDR471W	RPL27B	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl27Ap and has similarity to rat L27 ribosomal protein	**2**	**R, R**
YLR406C	RPL31B	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl31Ap and has similarity to rat L31 ribosomal protein; associates with the karyopherin Sxm1p	**2**	**R**
YER056C-A	RPL34A	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl34Bp and has similarity to rat L34 ribosomal protein	**3**	**RR**
YDL136W	RPL35B	Protein component of the large (60S) ribosomal subunit, identical to Rpl35Ap and has similarity to rat L35 ribosomal protein	**2**	**RR**
YMR194W	RPL36A	N-terminally acetylated protein component of the large (60S) ribosomal subunit, nearly identical to Rpl36Ap and has similarity to rat L36 ribosomal protein; binds to 5.8 S rRNA	**3**	**R**
YDR500C	RPL37B	Protein component of the large (60S) ribosomal subunit, has similarity to Rpl37Ap and to rat L37 ribosomal protein	**3**	**RR, RR**
YJL189W	RPL39	Protein component of the large (60S) ribosomal subunit, has similarity to rat L39 ribosomal protein; required for ribosome biogenesis; exhibits genetic interactions with SIS1 and PAB1	**3**	**R**
YNL096C	RPS7B	Protein component of the small (40S) ribosomal subunit, nearly identical to Rps7Ap; interacts with Kti11p; deletion causes hypersensitivity to zymocin; has similarity to rat S7 and Xenopus S8 ribosomal proteins	**1**	**RR**
YBR189W	RPS9B	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps9Bp and has similarity to E. coli S4 and rat S9 ribosomal proteins	**2**	**R**
YBR048W	RPS11B	Protein component of the small (40S) ribosomal subunit; identical to Rps11Ap and has similarity to E. coli S17 and rat S11 ribosomal proteins	**2**	**R**
YDL083C	RPS16B	Protein component of the small (40S) ribosomal subunit; identical to Rps16Ap and has similarity to E. coli S9 and rat S16 ribosomal proteins	**1**	**R**
YDR447C	RPS17B	Ribosomal protein 51 (rp51) of the small (40s) subunit; nearly identical to Rps17Ap and has similarity to rat S17 ribosomal protein	**1**	**R**
YDR450W	RPS18A	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps18Bp and has similarity to E. coli S13 and rat S18 ribosomal proteins	**1**	**R**
YDR083W	RRP8	Ribosomal RNA Processing: nucleolar protein required for efficient processing of pre-rRNA at site A2; methyltransferase homolog	**2**	**R**
YPL193W	RSA1*	RiboSome Assembly	**2**	**R**
YKR029C	SET3	NAD-dependent histone deacetylase activity	**2**	**RR**
YOR140W	SFL1	Suppressor gene for Flocculation: Transcription factor with domains homologous to Hsf1p and to myc oncoprotein; required for normal cell surface assembly and flocculence	**2**	**RRR**
YLR058C	SHM2	serine hydroxymethyltransferase	**3**	**R**
YBR103W	SIF2	Sir4p-Interacting Factor; 535 amino acid protein containing 4 WD-40 repeats and a nuclear localization signal	**2**	**RRR**
YGL213c	SKI8	essential for protection against viral cytopathology, dispensable for mitotic but required for meiotic recombination and spore viability; antiviral protein, mRNA is induced early in meiosis	**2**	**RRR**
YBL061C	SKT5	Activator of Chs3p (chitin synthase III), recruits Chs3p to the bud neck via interaction with Bni4p; has similarity to Shc1p, which activates Chs3p during sporulation	**3**	**RRR***
YMR216C	SKY1	Protein serine kinase with similarity to human SRPK1, which is a serine kinase that specifically phosphoryates arginine-serine rich domains found in the SR family of splicing factors	**2**	**R**
YBR156C	SLI15	Sythentically Lethal with Ipl1: Mitotic spindle protein involved in chromosome segregation	**2**	**R**
YBR266C	SLM6	Hypothetical ORF: Synthetic Lethal with Mss4	**3**	**RRR**
YCR033W	SNT1	NAD-dependent histone deacetylase activity	**2**	**RR**
YMR016C	SOK2	Protein that can when overexpressed suppress mutants of cAMP-dependent protein kinase; displays homologies to several transcription factors	**3**	**R**
YHR066W	SSF1	putative involvement in mating; homologous to Ssf2p	**3**	**R**
YDR443C	SSN2	Suppressor of SNf1: Required for stable association of Srb10p-Srb11p kinase with RNA polymerase holoenzyme; regulates YGP1 epxression; component of RNA polymerase II holoenzyme and Kornberg's mediator (SRB) subcomplex; transcription factor	**2**	**RR, R**
YGL232W	TAN1	Putative tRNA acetyltransferase, RNA-binding protein required for the formation of the modified nucleoside N(4)-acetylcytidine in serine and leucine tRNAs but not required for the same modification in 18S rRNA	**1**	**R**
YML014W	TRM9*	tRNA methyltransferase, catalyzes the esterification of modified uridine nucleotides in tRNAs, creating 5-methylcarbonylmethyluridine in tRNA(Arg3) and 5-methylcarbonylmethyl-2-thiouridine in tRNA(Glu); may have a role in stress response	**1**	**RRR***
YDR354W	TRP4	anthranilate phosphoribosyl transferase	**1**	**R**
YHR111W	UBA4	Protein that activates **Urm1p** before its conjugation to proteins (urmylation); one target is the thioredoxin peroxidase Ahp1p, suggesting a role of urmylation in the oxidative stress response	**3**	**R**
YKR042W	UTH1	Youth, involved in determining yeast longevity; involved in cell growth	**3**	**RR**

Appendix C: Zymocin resistant diploid deletion mutants in *S. cerevisiae*

YNR012W	URK1	converts ATP and uridine to ADP and UMP; Uridine kinase	**2**	**R**
YIL008W	URM1	ubiquitin-like protein	**2**	**RR**
YDR451C	YHP1	acts as a repressor at early cell cycle boxes (ECBs) to restrict their activity to the M/G1 phase of the cell cycle	**1**	**R**
YKL126W	YPK1	76.5 kDa Serine/threonine protein kinase with similarity to protein kinase C, is 90% identical to Ypk2p	**3**	**R**
YDR349C	YPS7	Putative GPI-anchored aspartic protease, located in the cytoplasm and endoplasmic reticulum	**3**	**RR**
YIR026C	YVH1	nitrogen starvation-induced protein phosphatase	**3**	**RRR**
YBL071C	ORF, Dubious	Hypothetical ORF	**3**	**RRR***
YDL041W*	ORF, Dubious	Hypothetical ORF	**2**	**RRR***
YDL062W	ORF, Dubious	Hypothetical ORF	**2**	**RR, R**
YDL063C	ORF	ORF, Uncharacterized	**2**	**RR, R**
YDR290W	ORF, Dubious	Dubious open reading frame, unlikely to encode a protein; not conserved in closely related Saccharomyces species; 42% of ORF overlaps the verified gene **RTT103**; deletion causes hydroxyuracil sensitivity	**2**	**RR**
YDR417C*	ORF, Dubious	Hypothetical ORF	**3**	**RRR,RRR***
YGL214W	ORF, Dubious	Dubious open reading frame, not conserved in closely related Saccharomyces species; deletion mutation enhances replication of Brome mosaic virus in S. cerevisiae, but this is likely due to effects on the overlapping gene **SKI8**	**2**	**R**
YML036W*	ORF	ORF, Uncharacterized	**3**	**R**
YMR003W	ORF	ORF, Uncharacterized	**3**	**RR**
YMR135W-A	ORF, Dubious	Hypothetical ORF	**2**	**R**
YNL109W	ORF	ORF, Dubious	**2**	**RR**
YNL120C	ORF, Dubious	Dubious open reading frame, not conserved in closely related Saccharomyces species; deletion mutation enhances replication of Brome mosaic virus in S. cerevisiae, but this is likely due to effects on the overlapping gene **NCS2**	**3**	**R**
YNL228W	ORF, Dubious	Hypothetical ORF	**3**	**RR**
YNL226W	ORF, Dubious	Hypothetical ORF	**3**	**RR**
YNR009W	ORF	ORF, Uncharacterized	**2**	**R**
YNR029C	ORF	ORF, Uncharacterized	**2**	**R**
YOR309C	ORF, Dubious	Hypothetical ORF	**2**	**R**
YOR345C	ORF, Dubious	Hypothetical ORF	**1**	**R**
YOR379C	ORF, Dubious	Hypothetical ORF	**1**	**R**

Gene* Gene deletion or ORF demonstrates cross sensitivity to ionizing radiation

Resistance: as determined after 2 days growth at 30°C during initial screening

1 = no growth on 66% zymocin plate, partial growth on 33% zymocin plate
2 = partial or no growth on 66% zymocin plate, uninhibited growth on 33% zymocin plate
3 = growth on 66% and uninhibited growth on 33% zymocin plate

Dilution Plating:

R = 5 fold enhanced resistance (compared to WT) following dilution plating on 66% zymocin plate
RR = 25 fold enhanced resistance following dilution plating on 66% zymocin plate
RRR = 125 fold (or greater) enhanced resistance following dilution plating on 66% zymocin plate

RRR* deletion shows no growth inhibitory or lethal effects of zymocin (identical to growth on YPD)

BRCA1 INTERACTS WITH HIGHLY CONSERVED COMPONENTS OF THE TRANSCRIPTION ELONGATOR COMPLEX

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The tumor suppressor protein BRCA1 has been implicated in a wide variety of DNA metabolic functions that maintain genome integrity including DNA damage signaling, checkpoint activation, transcription, centrosome duplication and recombination. To elucidate a fundamental functional role for BRCA1, we selected diploid Saccharomyces. cerevisiae deletion mutants that suppressed the G1 arrest and lethality observed following heterologous expression of BRCA1. A high copy, selectable (URA3) plasmid containing BRCA1 fused to the galactose promoter was transformed into a pool of 4746 diploid deletion strains each carrying a unique 20bp molecular tag. Transformed cells were immediately plated to synthetic complete galactose - uracil medium (BRCA1 expressed) and rapidly growing mutant colonies were isolated. Deletion strains were identified by sequencing the 20bp tag and BRCA1 expression was confirmed by immunofluorescence or Western blot analysis. We also screened our collection of ionizing radiation (IR) sensitive diploid deletion strains for those that could suppress the BRCA1-induced G1 arrest and lethality. From these screens we independently identified highly conserved interactive components of the CCR4 damage response network as well as factors that appear to participate in transcription elongation. The majority of these genes, including CCR4, DHH1, DEF1, HCM1, SPT4, SPT5, SUB1, YAF9 and numerous components of the nuclear pore complex are required for transcription and confer resistance to IR as well as the transcription elongation inhibitors mycophenolic acid (MPA) and/or zymocin. Furthermore, when these genes are deleted, they mediate a prolonged G1 cell cycle arrest following DNA damage but conversely allow rapid G1/S cell cycle transition following BRCA1 expression. Moreover, nonlethal doses of transcription elongation inhibitors greatly augmented BRCA1-induced lethality in WT yeast. We propose that in WT yeast, BRCA1 binds to elongating transcription complexes and stalls mRNA elongation in G1. Alternatively, transcription elongation may stall when DNA damage is encountered. Eventually, cells adapt to this persistent DNA damage and enter S phase where the stalled transcription complexes serve as replication blocks that are processed into lethal DNA double-strand breaks. Consistent with this idea is the observation that BRCA1 induces significantly enhanced plasmid degradation and loss in WT as compared to SPT4 deleted cells. Using co-immunoprecipitation, we determined that Ccr4p and Dhh1p physically interact with BRCA1 in yeast, while the highly conserved human orthologue of Dhh1p (DDX6) physically interacts with BRCA1 in human cells. The formation of immunofluorescent BRCA1 foci following treatment of human MCF7 cells with MPA strongly suggests that BRCA1 similarly interacts with stalled elongating transcription complexes at sites of DNA damage in human cells. Thus we have successfully used yeast as a functional genomic "tool" for the rapid identification of a novel, highly conserved mRNA damage surveillance pathway in which BRCA1 plays a critical role.